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LARYNGEAL SYNKINESIS: SIGNIFICANCE TO THE LARYNGOLOGIST

BOTULINUM TOXIN INJECTION

ACUTE MANAGEMENT OF LARYNGEAL TRAUMA

LASER SURGERY FOR CARCINOMA INVOLVING ANTERIOR COMMISSURE

CHOANAL ATRESIA: MANAGEMENT TRENDS

ISOLATED PULMONARY NODULES IN HEAD AND NECK CANCER PATIENTS

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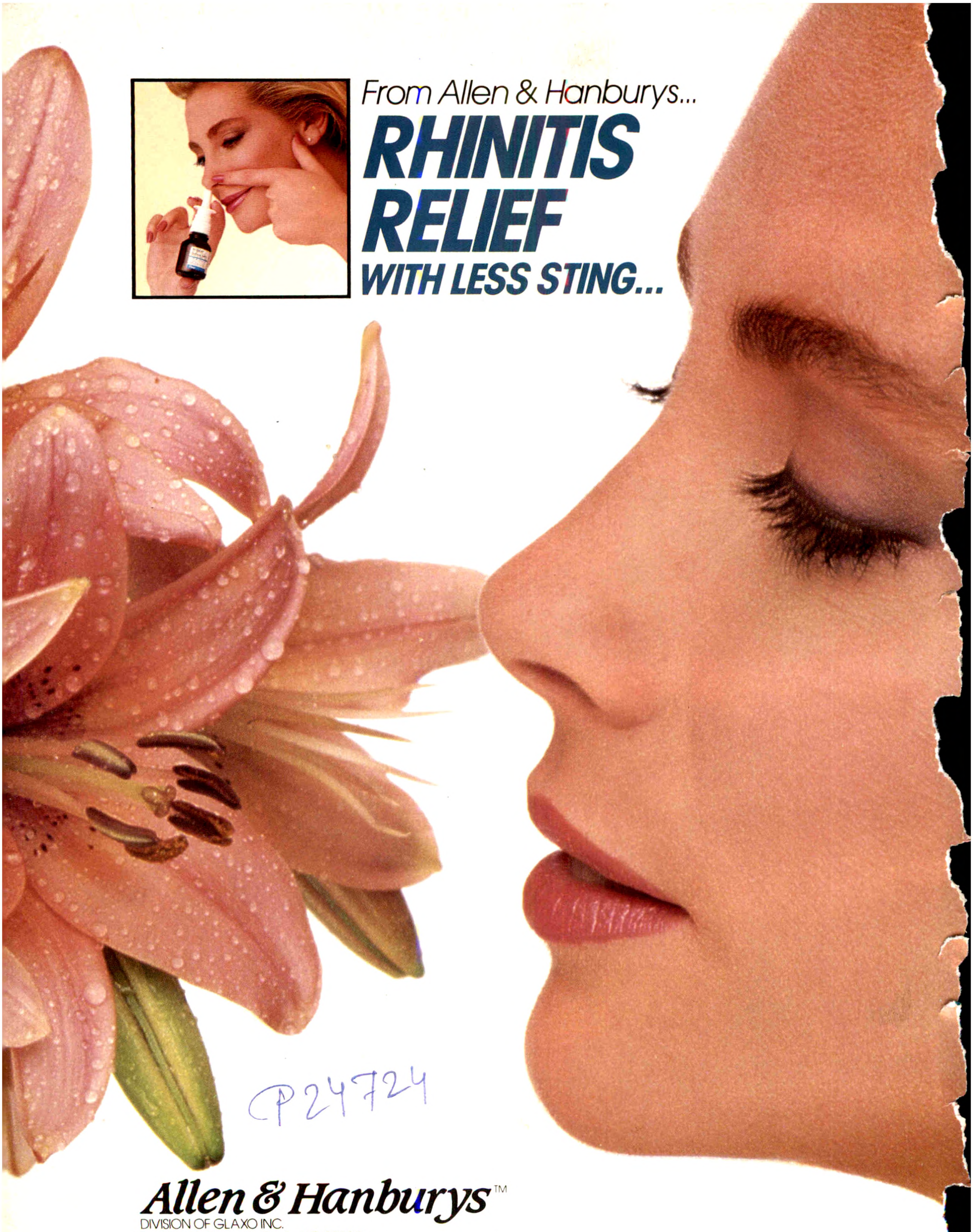
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LARYNGEAL SYNKINESIS: ITS SIGNIFICANCE TO THE LARYNGOLOGIST

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Basic research and surgical cases have shown that the injured recurrent laryngeal nerve (RLN) may regenerate axons to the larynx that inappropriately innervate both vocal cord adductors and abductors. Innervation of vocal cord adductor muscles by those axons that depolarize during inspiration is particularly devastating to laryngeal function, since it produces medial vocal cord movement during inspiration. Many patients thought to have clinical bilateral vocal cord paralysis can be found to have synkinesis on at least one side. This will make the glottic airway smaller, particularly during inspiration, than would true paralysis of all the intrinsic laryngeal muscles. Patients with bilateral vocal cord paralysis should undergo laryngeal electromyography. If inspiratory innervation of the adductor muscles is present, simple reinnervation of the posterior cricoarytenoid muscle will fail. The adductor muscles also must be denervated by transection of the adductor division of the regenerated RLN.

KEY WORDS — recurrent laryngeal nerve, synkinesis, vocal cord paralysis.

INTRODUCTION

The neural control of the larynx is far more complicated than is recognized by most clinicians. The recurrent laryngeal nerve (RLN) contains from 500 to 1,000 motor axons, most of which pass to the adductor muscles (lateral cricoarytenoid [LCA], interarytenoid [IA], and thyroarytenoid [TA]). Approximately one fourth of the fibers are destined for the posterior cricoarytenoid (PCA) muscle.[1] Each RLN is also thought to contain fusimotor efferent axons as well as vasomotor and secretomotor fibers of sympathetic and parasympathetic origin.[1] In addition, each RLN supplies sensory innervation to the subglottic larynx and trachea.

When an RLN is cut, crushed, stretched, or otherwise damaged, neural regeneration of some degree usually occurs. This axon regrowth may fail to reach the paralyzed laryngeal muscles, or it may achieve the desired reinnervation of the laryngeal muscles, or it may produce aberrant regeneration. This aberrant or misdirected regeneration to the laryngeal muscles produces synkinesis, the subject of this report.

Synkinesis can be defined as the synchronous contraction of muscles that do not normally contract together. It is well known in facial nerve diseases and injuries, and several mechanisms have been proposed and elucidated.[2] Laryngeal synkinesis is the misdirected and inappropriate reinnervation of abductor laryngeal muscles by adductor fibers of the RLN, and vice versa. This results in glottic neuromuscular dyscoordination, if not chaos, during the respiratory cycle. Medial bulging and spasms of the TA muscle during inspiration may cause glottic impairment and airway obstruction.[3-5] Synkinetic reinnervation, which results from inappropriate innervation of the TA, IA, and/or LCA muscles by abductor axons, produces medial rotational forces on the arytenoid during inspiration, and effectively nullifies any appropriate lateral abductive simultaneous contractions by the PCA muscle.[5] Conversely, PCA muscle innervation by previous adductor (phonatory, expiratory) axons produces attempts at lateral arytenoid rotation that are effectively counteracted by simultaneous opposing, and stronger, contractions of the LCA, IA, and TA muscles during phonation.[1]

Laryngeal synkinesis is well known in veterinary medicine, having been documented as one of the abnormal findings in subclinical equine vocal cord paralysis, a laryngeal disorder in horses associated with a condition known as "roaring."[7]

A prior study by Crumley and McCabe demonstrated the regenerating RLN's capabilities for synkinesis, and also proved that the RLN had strong regenerative capabilities.[8] In this study, it was shown that RLN axons in a dog could grow through and/or around silk ligatures tied on the RLN stump in an attempt to prevent nerve regeneration. Abductor and adductor reinnervations were unpredictable and random. Some animals attained only abductor reinnervation, while others demonstrated only adductor reinnervation, and others had both. The inability to control this random and inappropriate nerve regeneration best explains clinical cases of respiratory distress following RLN sectioning.[9]

Given this propensity for the RLN to regenerate in a random fashion, it is reasonable to assume that

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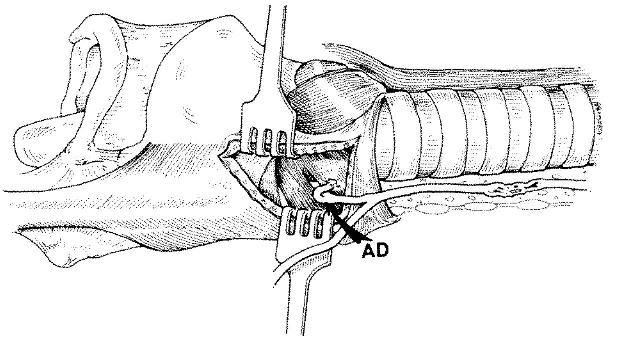

Fig 1. (Case 1) Diagram of operation performed. Vocal cord adductors had become reinnervated, while posterior cricoarytenoid (PCA) muscle was paralyzed. Adductor division (AD) of recurrent laryngeal nerve was transected and implanted into PCA muscle with intention of denervating adductor muscles and reinnervating PCA muscle.

certain cases of vocal cord paralysis, as diagnosed by the clinician, are actually instances wherein the abductor and adductor muscles on the "paralyzed" side are simultaneously contracting. The laryngologist, seeing no movement of the vocal cord, makes the clinical diagnosis of "vocal cord paralysis." If such reinnervation were partial and limited it could yield, in cases of unilateral vocal cord paralysis, a voice that might sound nearly normal. In these instances the cord would not appear to abduct and adduct (and in fact usually would appear immobile), yet the voice would be surprisingly good. (This is also seen when the abductors and adductors are innervated in a random fashion by ansa hypoglossi anastomosis to the entire RLN.)[10]

Hence when RLN regeneration results in synkinesis following unilateral paralysis, the laryngologist may detect subtle trembling movements in the various intrinsic laryngeal muscles, and see a vocal cord in the paramedian position. The phonatory quality ordinarily will be better than that observed in long-standing and complete unilateral vocal cord paralysis. If carefully looked for, these subtle fasciculations are not infrequently observed in unilateral and bilateral vocal cord paralysis (BVCP).

Patients with clinical BVCP (which could be paralysis on one side with paresis on the other, or a combination of synkinesis with paresis or paralysis) present a different situation. In these patients, if laryngeal synkinesis (adductor and abductor reinnervation by both adductor and abductor RLN fibers) is present, the vocal cord may be more medial and under more tension than if the intrinsic muscles remained truly paralyzed, eg, partial synkinetic reinnervation of the adductor and abductors on both sides in a patient with BVCP may result in a smaller glottic airway than in cases in which both sides remained entirely paralyzed. This is largely due to LCA and TA muscle innervation by inspiratory-firing axons. In addition the innervated PCA muscle contracts to stabilize the arytenoid and counteract the cricothyroid (CT) muscle's anterior pull on the vocal process and vocal ligament, such as when the intact PCA muscle contracts during high-pitched phonation.[11,12] This PCA muscle activity paradoxically may enhance the adductive function of the CT muscle in RLN paralysis, further impairing glottic function.

CASE REPORTS

Case 1. A 31-year-old man was involved in a motorcycle accident. An endotracheal tube was placed for several weeks. Upon extubation he was found to have a left vocal cord paralysis. Two years later he developed Guillain-Barré syndrome, necessitating tracheotomy. Following recovery and decannulation, he had moderate inspiratory stridor. Indirect laryngoscopy revealed BVCP. He did not have a tracheotomy and did not desire to have one. His major wish was for more exercise tolerance.

Laryngeal reinnervation procedures were discussed with him, as well as were arytenoidectomy, tracheotomy, and arytenoidopexy procedures. It was agreed that he undergo left RLN exploration, and if the operating team felt that it was feasible, to proceed with a reinnervation procedure. (He was not a candidate for phrenic-abductor branch anastomosis as the Guillain-Barré syndrome had significantly impaired both diaphragms' excursions.)

At operation, the left RLN was found and dissected superiorly into the larynx. Intraoperative nerve stimulation studies were done with simultaneous direct laryngoscopy. Stimulation of the adductor division (with stimulus frequencies from 20 to 60 Hz) produced strong adduction, while stimulation of the abductor branch produced no vocal cord abduction or visible PCA muscle contraction. The adductor division of the RLN was divided and implanted into the PCA muscle (Fig 1). Postoperatively there was immediate subjective airway improvement, although it was accompanied by minor deterioration in phonatory quality.

Case 2. A 14-year-old boy had had a series of thoracic and cervical surgical procedures for tracheoesophageal fistula, which resulted in BVCP. When first seen by this author, both cords appeared to be paralyzed in the paramedian position. Following direct laryngoscopy with the microscope, there appeared to be fine trembling movements of the right arytenoid and aryepiglottic fold.

In reviewing the details of his last surgical procedure, performed by a pediatric surgeon, it was ascertained that the right RLN had not been dissected, and in fact the cord paralysis was somewhat of a surprise. This raised the question of a cautery, stretch, or clamp injury (each of which might allow RLN regeneration and synkinesis, as opposed to RLN transection or avulsion.)

Electromyography (EMG) revealed laryngeal synkinesis (Fig 2A,B). Both the LCA and TA mus-

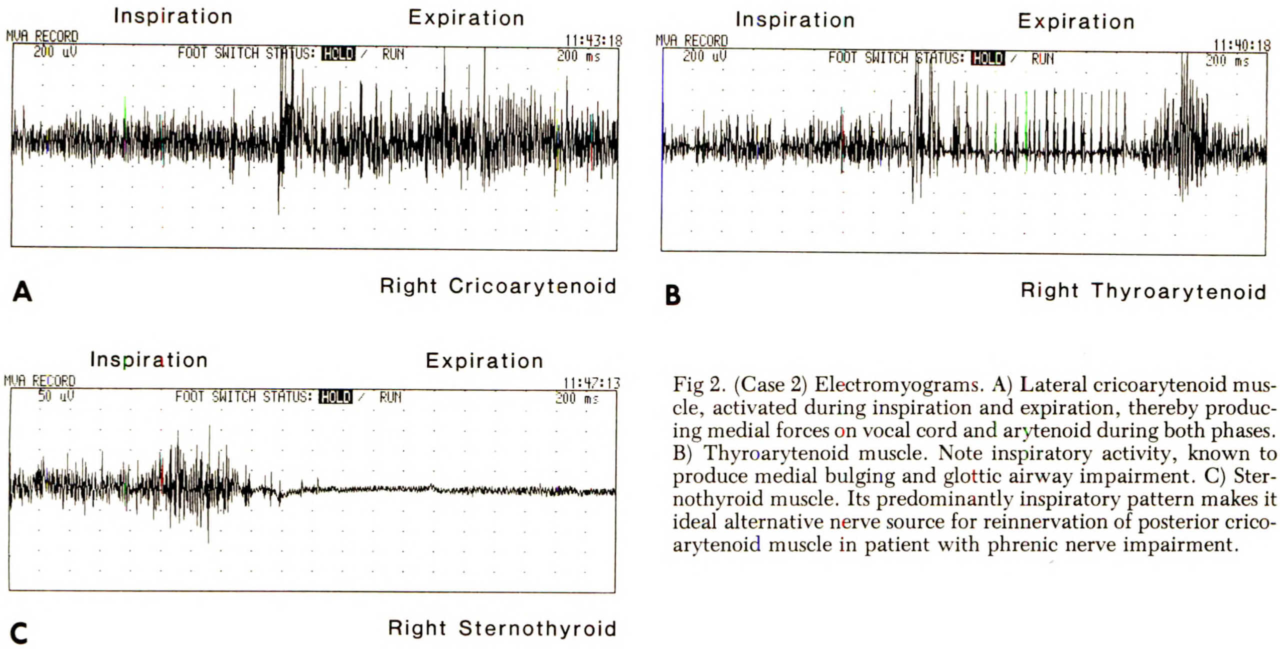

Fig 2. (Case 2) Electromyograms. A) Lateral cricoarytenoid muscle, activated during inspiration and expiration, thereby producing medial forces on vocal cord and arytenoid during both phases. B) Thyroarytenoid muscle. Note inspiratory activity, known to produce medial bulging and glottic airway impairment. C) Sternothyroid muscle. Its predominantly inspiratory pattern makes it ideal alternative nerve source for reinnervation of posterior cricoarytenoid muscle in patient with phrenic nerve impairment.

cles showed synkinetic changes, with electrical activity in both expiration and inspiration. Since the phrenic nerve was thought to be damaged on the right side (as in case 1), phrenic-to-abductor branch RLN anastomosis was not judged to be feasible. Accordingly, EMG confirmation of normal innervation (inspiratory pattern) of the sternothyroid (ST) muscle was obtained (Fig 2C). The ST muscle, which normally depolarizes in inspiration, was found to be normally innervated, and consequently thought to represent an appropriate source of purely inspiratory nerve stimulation for the abductor muscle.

The RLN was explored. Both abductor and adductor components were synkinetic, as seen by direct laryngoscopy during intraoperative nerve stimulation studies. Stimulation at all frequencies of the adductor division produced adduction. Stimulation of the abductor branch to the PCA muscle produced abduction. This confirmed that regeneration had occurred into both adductor and abductor branches of the RLN and, combined with the EMG data, proved the presence of synkinetic reinnervation.

The adductor division was cut and implanted into the PCA muscle. The PCA branch was left intact. Thus all axons that would regenerate up the RLN were routed to the PCA muscle (Fig 3). (This approach would have effectively routed all regenerating axons within the RLN to the PCA muscle, producing tonic abduction throughout the respiratory cycle.) Since this would have lateralized the arytenoid and vocal cord in both phases of respiration, we elected to transect the RLN proximally, and innervate the PCA muscle (via this RLN conduit) with purely inspiratory axons from the ST muscle's nerve. Accordingly, the RLN was divided proximally (below the cricoid cartilage), and then the nerve to the ST muscle (inspiratory muscle) was sutured to the RLN at the level of the second tracheal ring. Thus the RLN segment from its anastomosis with the ST nerve below, to its insertion into the PCA, served as a conduit for purely inspiratory neural control for the abductor muscle.

Another small nerve branch from the ansa hypoglossi was then anastomosed to the distal adductor division of the RLN, in hopes of obtaining noncompetitive innervation of the adductor muscles and retention of the satisfactory preoperative voice.

Postoperatively the cord was obviously paralyzed and appeared more lax, evidence that the RLN transection had paralyzed a synkinetic vocal cord. The voice was weaker and definitely more breathy. At 2 months the airway was improved. At 4 months the voice was reportedly stronger, abductive movements were seen during inspiration, and airway improvement persisted.

DISCUSSION

The frequency of laryngeal synkinesis following RLN injury is unknown. Duncan and Baker[13] found that eight of 12 ponies having experimental RLN crush injuries developed trembling and asynchronism of the cords' movements. In experiments in the dog by Crumley and McCabe,[8] six of eight animals demonstrated synkinesis following RLN transection and ligation. It is assumed that stretching, certain cauterization injuries, and other injuries in which the nerve is not totally transected would increase the possibility for regeneration and subsequent synkinesis.

It is known that the abductor branch of the RLN is a characteristic slow-twitch motor nerve. Most of

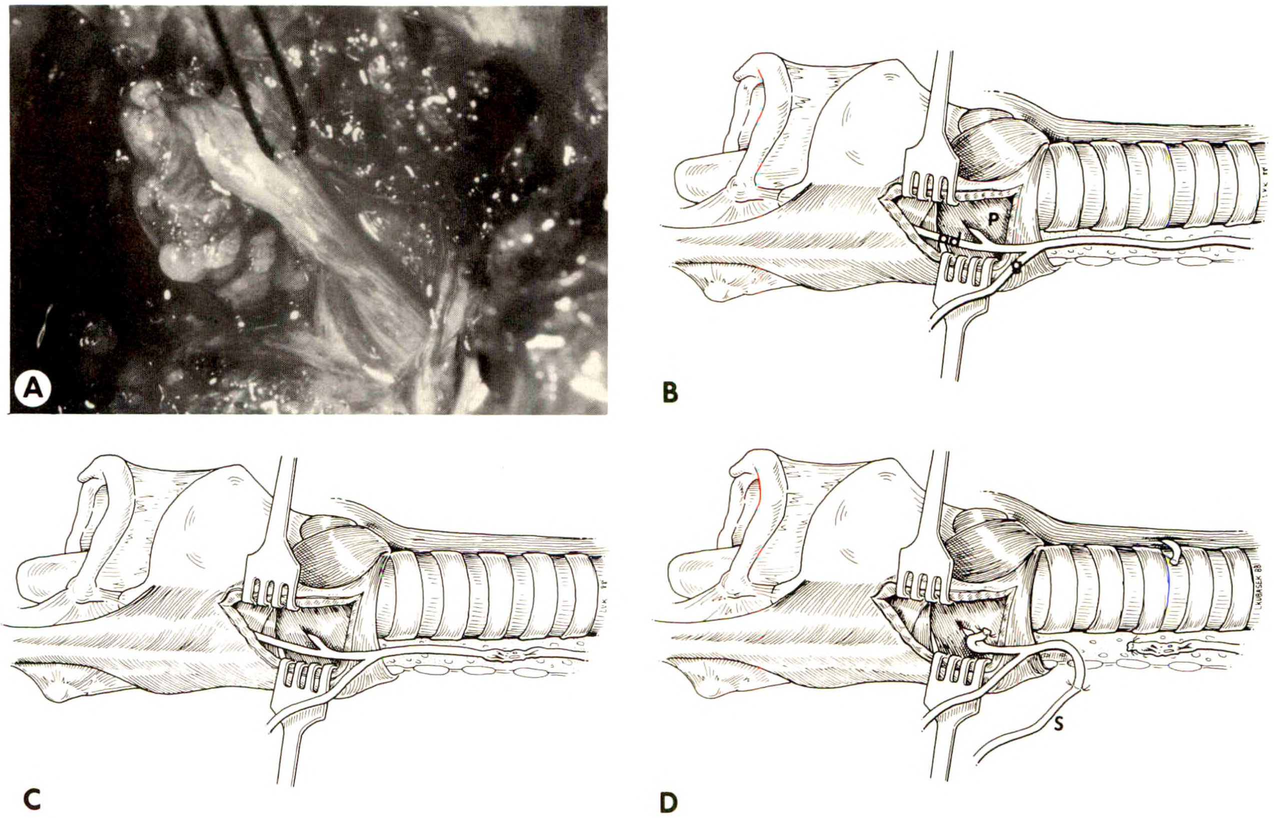

Fig 3. (Case 2) A) Photograph of recurrent laryngeal nerve (RLN) with silk ligature around tiny branch to posterior cricoarytenoid (PCA) muscle, which when stimulated produced pure abduction. B) Diagram of same. p — abductor branch and PCA muscle, ad — adductor division of RLN, g — Galen's anastomosis. C) Synkinetic reinnervation of both adductors and abductor was present, because of aberrant regeneration in RLN distal to injury. (Inspiratory and expiratory nerve fibers innervated both adductor and abductor muscles.) D) Ansa branch to sternothyroid (S) muscle anastomosed to distal RLN. Adductor division transected and implanted into PCA muscle.

the axons in this branch contain 200 to 250 muscle fibers in each motor unit.[14] This is in contradistinction to the fast-twitch fibers in the adductor division of the RLN with motor unit sizes of two to 20 muscle fibers.[15] After a nerve transection, when both fiber types have an opportunity to regenerate back toward the larynx, it is possible that one type regenerates faster and might have an advantage in regeneration versus the other type, by reaching the paralyzed muscles first and securing motor end plate connections. This is unknown at this time.

What is known is that the inspiratory slow-twitch axons (formerly abductor to PCA muscle, with larger motor units) may reinnervate many more muscle fibers than can the more numerous fast-twitch fibers. This may give the abductor fibers (formerly to PCA muscle) an advantage in securing motor end plates and producing reinnervation, irrespective of whether the regenerating axons were in abductor or adductor muscle.

When these slow-twitch fibers do reinnervate the TA muscle, they are known to produce medial bulging and mounding of the vocal cord during inspiration, a phenomenon that may produce glottic airway obstruction.[4,5,16,17] This mechanism would obviously increase airway impairment in patients with suspected BVCP.

These data have profound therapeutic implications. In unilateral RLN injuries, some patients may have return of normal laryngeal function, particularly if the nerve ends have not been separated, or if the nerve has been merely bruised, temporarily clamped, or stretched. Since some synkinetic larynges ultimately may demonstrate satisfactory phonatory voices, Teflon should not be injected for 1 year following such injuries. Rather, Gelfoam paste (or Zyderm collagen, which lasts longer than Gelfoam) will allow the regenerating RLN its optimum chance to achieve this reinnervation, producing a phonatory end result far superior to that with Teflon injection. Electromyography can be done readily during this time period to follow the course of nerve regeneration and reinnervation.

In BVCP, since both sides are afflicted, there is double the chance that synkinesis may affect the final result. If synkinesis is present on either side, it is probable that the involved vocal cord is positioned more medially (and hence the glottic airway is smaller) than it would be were the intrinsic muscles actually paralyzed.

Consequently, in patients with BVCP, both sides of the larynx should be investigated with EMG. If synkinesis is found (as evidenced by inspiratory innervation of adductor or phonatory muscles, eg, TA or LCA muscles), then consideration should be given to RLN exploration and intraoperative stimulation studies with simultaneous direct laryngoscopy. It is important to vary the stimulator's frequency, since DC stimuli below 20 pulses per second will excite preferentially only slow-twitch fibers, while frequencies over 40 pulses per second will excite fast-twitch fibers.[5]

If laryngeal synkinesis is found, selective reinnervation of the PCA muscle will fail, since the stronger adductor muscles will continue to adduct the cord during inspiration, even if PCA muscle reinnervation is successful. Consideration therefore should be given to transecting both the adductor division and abductor branch, prior to any reinnervation procedure for the PCA muscle.

If a new source is not to be used, such as the phrenic transfer or neuromuscular pedicle, it is possible that transection (alone) of the adductor division will improve the airway.[4,5,17,18] The adductor division, since it contains four fifths of the RLN's motor fibers,[7] should be sutured to the distal end of the abductor branch, in order to route more axons and stimuli to the PCA muscle, and less to the adductors, as described by Doyle et al.[18] The cut proximal end of the abductor branch should be implanted into the PCA muscle as well, to optimize chances for regenerating axons in the smaller abductor branch to also reinnervate abductor muscle fibers. If an operative approach is not desired, injection of botulinum A into the adductor muscles may be considered, although this would only provide temporary alleviation of the synkinesis.

In synkinetic larynges during inspiration the PCA muscle attempts to abduct the arytenoid at the same time as the stronger adductor muscles rotate the vocal process medially. The retained innervation of the PCA muscle stabilizes and, in fact, rocks the arytenoid somewhat posteriorly, giving the CT muscle a stronger fulcrum posteriorly against which to pull the vocal ligament. This may force the vocal cord even further medially.[19]

In all patients with BVCP, whether synkinetic reinnervation is present or not, it is known that the CT muscle's reflex activity results in a decreased glottic aperture during inspiration. This is of course because the CT muscle is an accessory inspiratory muscle, acting to lengthen the glottis in the anteroposterior diameter during inspiration in normal individuals.[20,21] Since this normal CT muscle inspiratory contraction causes some stretching of the vocal cord (and medialization when the PCA muscle is paralyzed), it is interesting that CT muscle contraction is increased by respiratory obstruction.[20] Since the paralyzed PCA muscle does not abduct the arytenoid, this reflex increase in CT contraction further medializes the paralyzed cord and decreases the glottic aperture.[20] Although CT nerve transection and CT muscle injection with botulinum toxin have been advocated for airway improvement in BVCP, recognition and operative treatment of laryngeal synkinesis offer more opportunity for normal laryngeal function and retention of normal CT muscle function.

SUMMARY

Recognition of laryngeal synkinesis and its mechanisms of action is vital in the diagnosis and management of BVCP. While further research is needed, it it clear that the anatomic proximity that exists in the larynx between two diametrically opposed muscle groups (vocal cord adductor and abductor [agonist–antagonist]) allows for this neuromuscular mechanism to have far more critical effects than anywhere else in the body.

Attempts at reinnervation of the PCA muscle for BVCP will not succeed when inspiratory innervation of the adductor muscles exists. Knowledge of these mechanisms will allow the laryngologist to more appropriately treat patients with both unilateral vocal cord paralysis and BVCP.

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NINDS NOTES*

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The National Institute of Neurological Disorders and Stroke, along with the National Institute on Child Health and Human Development and the National Institute of Mental Health, invites grant applications for research on the brain, immune system, and neurobehavioral aspects of human immunodeficiency virus (HIV) infection. Studies are particularly encouraged that examine

- HIV in the nervous system and its effects on behavior, including AIDS dementia, and learning and memory deficits
- the role of repeated stressful events on immune function
- neurologically mediated behavioral influences on immune response.

Applicants may request support for up to 5 years through one of several grant mechanisms, including the First Independent Research Support and Transition (FIRST) award and the program project award. Application receipt deadlines are January 2, May 1, and September 1.

For further information, contact Dr A. P. Kerza-Kwiatecki, Division of Demyelinating, Atrophic, and Dementing Disorders, 7550 Rockville Pike, Rm 708, NINDS, NIH, Bethesda, MD 20892; (301) 496-1431.

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BOTULINUM TOXIN INJECTION FOR THE TREATMENT OF OROMANDIBULAR DYSTONIA

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STANLEY FAHN, MD
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Dystonia is a neurologic disorder characterized by abnormal, involuntary movements causing twisting and turning postures; it is postulated to be a disorder of central motor processing. The dystonias, when classified by region of the body involved, have been characterized as focal, segmental, and generalized. Focal dystonia can affect jaw mechanics, leading to forceful contraction of the jaw muscles and resulting in inappropriate deviation of the jaw. Localized injections of botulinum toxin have been used successfully in the management of other focal or segmental dystonias. We have treated 20 oromandibular dystonia patients with botulinum toxin. Six patients had only jaw and tongue involvement; 11 had blepharospasm and jaw involvement; and three had jaw involvement as part of a more generalized dystonia. Five patients had been diagnosed originally and treated as having temporomandibular joint syndrome. All but one of the patients had improvement of their symptoms with the toxin injections. The patients averaged 47% improvement with the injections.

KEY WORDS — botulinum toxin, oromandibular dystonia.

INTRODUCTION

In 1899 Glowers[1] described conditions producing tonic and clonic jaw contractions. Causes of tonic spasms included tetanus, trauma, hysteria, pontine lesions, and exposure to cold. Clonic spasms were described with convulsions, rigors, paralysis agitans, facial pain, and chorea.

In 1910 Meige[2] described a syndrome of spasms of the eyes as well as contractures of the pharynx, jaw muscles, and muscles of the floor of the mouth and tongue. Meige described muscle spasms of prolonged duration with an irregular, repetitive pattern. These spasms were typically provoked by voluntary action such as talking or eating. Sometimes the symptoms would be relieved or lessened by lying down, humming, singing, yawning, chewing, blowing air, or opening the mouth. A small number of these patients with Meige's syndrome develop torticollis and/or writer's cramp as more generalized features of their dystonia. Marsden[3] reported in 1976 that blepharospasm-oromandibular syndrome was part of a group of adult-onset segmental torsion dystonias.

We now know that involuntary movements of the jaw are seen in tardive dyskinesias, idiopathic dystonia, secondary dystonia, acute dystonic reactions to neuroleptic drugs, tardive dystonias, dystonia induced by L-dopa, and encephalitic parkinsonism[4,5] (Table 1). Bulbar palsies also have been found to produce trismus and/or bruxism. Some unusual masticatory spasms can be found in epileptic disorders, particularly focal motor epilepsy. Jankovic and Patel[5] recently described cases of Meige's syndrome secondary to upper brain stem strokes or to demyelination from multiple sclerosis. There also appears to be a genetic predisposition in approximately one fifth of the cases.

In 1968 Schwartz and Chayes[6] reported a condition termed "temporomandibular joint pain-dysfunction syndrome." The main complaint of the patient with this syndrome was pain in the head or face that would continue day and night for days to months with varying intensity. The condition is due

TABLE 1. CLASSIFICATION OF DYSTONIA

- Primary causes
  - Without hereditary pattern
  - With hereditary pattern
    - Autosomal dominant
    - Autosomal recessive
    - X-linked recessive
    - Undefined
- Secondary causes
  - Associated with other hereditary neurologic disorders (eg, Wilson's disease, Huntington's disease, neuronal ceroid lipofuscinosis, progressive supranuclear palsy, Hallervorden-Spatz disease, olivopontocerebellar atrophy, acquired hepatocerebral degeneration, Tourette's disease)
  - Environmental
    - Posttraumatic
    - Postinfectious
    - Vascular
    - Tumor
    - Toxic
    - Antipsychotic drugs (phenothiazines, piperazines, butyrophenones, thioxanthenes)
    - Antiemetics (prochlorperazine, promethazine, metachlopramide)
    - Antiparkinson agents (L-dopa, bromocriptine, lisuride, pergolide)
  - Dystonia associated with parkinsonism
  - Psychogenic dystonia

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TABLE 2. OROMANDIBULAR DYSTONIA — SUMMARY OF PATIENT DATA

Total patients	20
F	17 (85%)
M	3 (15%)
F/M ratio	5.7:1
Age range, F	28-85 yr
Mean age, F	60.7 yr
Age range, M	45-67 yr
Mean age, M	53.3 yr
Mean duration of symptoms, F	7.2 yr
Mean duration of symptoms, M	5.3 yr
Positive family history, F	2 (11.7%)
Positive family history, M	0 (0.0%)
No. of patients with history of treatment for temporomandibular joint syndrome	5 (25.0%)
No. of patients with Meige's syndrome	10 (50.0%)
Improvement	
0%-10%	7 (35%)
10%-20%	1 (5%)
50%	3 (15%)
75% or greater	9 (45%)
Total improvement in series	47%
M	32%
F	50%

to overactivity of the masticatory muscles associated with either grinding or clenching of the teeth. The result of the activity can be not only pain but spasms of the muscles and abnormal jaw positions in some patients with trismus. There may be considerable clicking and crepitus of the temporomandibular joint (TMJ) with deviation of the jaw when the mouth is opened.

The diagnosis of oromandibular dystonia (OMD) often can be challenging because of its rarity and unusual features, and early misdiagnosis can be common. In the series of Thompson et al,[7] almost all of the patients previously had been treated for bruxism with use of dental appliances; these were found to be of little value. Sometimes dental appliances were found useful for treating orofacial dyskinesias, as in the series of Sutcher et al.[8] Drug therapy can be useful in some patients with dystonia. The drugs that seem to change the symptoms of dystonia either enhance or decrease central neurotransmitter function. Anticholinergics and benzodiazepines bring mild to moderate relief in some patients.[9-11] Although pharmacotherapy is used for treatment of dystonia, the results are suboptimal in many patients. Patients with OMD refractory to pharmacotherapy have been referred to us for treatment with injections of botulinum toxin (BOTOX).

MATERIALS AND METHODS

We treated more than 239 patients with focal dystonia with BOTOX; the group included 20 patients with OMD alone or with other dystonic involvement. Previously we described some of our patients with a variety of focal dystonias,[12,13] including those of the larynx,[14] treated with local injections of BOTOX.

A written informed consent form as approved by our Institutional Review Board was signed by all patients. Most of the patients had failed a variety of pharmacologic trials. These patients first were evaluated with a standard history and physical examination that included a full neurologic evaluation and a head and neck examination.

Lyophilized BOTOX A (Oculinum) was obtained and stored frozen at −20°C until reconstitution with sterile saline (without preservative) at the time of injection. The quantity of toxin is expressed in units, with 1 unit representing the median lethal dose for mice. For the typical injection a dilution was performed to a final concentration of 2.5 U/0.1 mL.

Injection Technique. Injections were individualized for each patient. At the beginning of this study, there were no published standard guidelines for the treatment of focal dystonias, including OMD. In general our approach was empirical, beginning with small doses and titrating them to the needs of the patient, selecting the muscles that seemed to have the greatest spasm, and drawing upon our previous experience. All injections in this study were given to ambulatory patients in the Dystonia Research Center. In general, doses were divided into 10-unit (0.4 mL) aliquots with doses of 10 to 40 units (0.4 to 1.6 mL) per muscle. A tuberculin syringe with a 25-gauge monopolar Teflon-coated hollow electromyographic (EMG) needle was used for the injections. The injections of the temporalis and masseter muscles were performed percutaneously. The internal and external pterygoid muscles were injected transorally with EMG control for optimum placement of the toxin.

Follow-up. The patients were asked to call within the first week after injection and were examined and videotaped at 2 weeks after injection. Typically, the effect of the toxin takes place within the first 24 to 72 hours. Most of the patients noticed a general decrease in the strength of the spasms and often a correction of the jaw position. In many cases multiple injections of the masticatory muscles were necessary to achieve the optimum effect. These, in general, were carried out on a 1- to 4-week basis. Once the muscles involved and a dose response for each patient were discovered, injections needed to be given every 2½ to 4 months.

There are no validated, quantitative rating scales for functional capability in OMD. Therefore, during each patient contact, the patients rated themselves for functional activity—from 0%, totally disabled, to 100%, totally normal. In addition, the physicians made a similar rating and the most conservative rating was used as the percent improvement in all cases. Tables 2 and 3 include all patient data.

TABLE 3. OROMANDIBULAR DYSTONIA — INDIVIDUAL PATIENT DATA

Pt	Age (yr)/ Sex	Duration of Symptoms (yr)	Muscles Injected	Improvement (%)	Previous Treatment*	Other Muscles, Other Diseases	Previous TMJ Treatment
1	46 F	6	Ext pterygoids, digastric, int pterygoids, genioglossus, hyoglossus	75	Clonazepam, baclofen, imipramine, carbamazepine, anticholinergic	Polyneuritis, postanoxia, facial contracture, tongue involvement, left leg	–
2	54 F	5	Masseters, temporalis, genioglossus	0-10	Anticholinergic, baclofen, dopaminergic, carbamazepine, clonazepam, antidopaminergic	Tongue, lower face	+
3	60 F	2	Masseters, temporalis	75	Anticholinergic	Tongue	+
4	60 F	5	Temporalis, masseters, platysma	0-10	Anticholinergic, baclofen, dopaminergic, antidopaminergic	Upper face, lower face, arms, neck	–
5	62 F	6	Masseters	75	Baclofen, clonazepam, antidopaminergic	Tardive dystonia, upper face, lower face, tongue	–
6	68 F	5	Masseters, int pterygoids, ext pterygoids, orbicularis oris	50	Anticholinergic, dopaminergic, Sinemet, trihexyphenidyl, pergolide, bromocriptine	Parkinson's disease	–
7	76 F	16	Masseters, temporalis, platysma	0-10	Baclofen	Upper face	–
8†	28 F	21	Masseters, temporalis, orbicularis oris	50	Baclofen, anticholinergic, clonazepam, dopaminergic, antidopaminergic, carbamazepine	Generalized dystonia	–
9	48 M	4	Masseters, temporalis	10-20	Baclofen, anticholinergic	Upper face, lower face, neck	–
10	68 F	5	Masseters, temporalis, int pterygoids, ext pterygoids	50	Baclofen, clonazepam, anticholinergic, dopaminergic, antidopaminergic, carbamazepine		+
11	64 F	10	Masseters, temporalis, orbicularis oris	0-10	Baclofen, clonazepam, anticholinergic, dopaminergic, antidopaminergic, carbamazepine	Upper face, lower face, tongue	–
12	49 F	3 mo	Masseters, temporalis	75	Clonazepam	Upper face, lower face, tongue, neck	–
13	45 M	2	Masseters, ext pterygoids	75	Clonazepam, baclofen	Secondary to local trauma	+
14	67 M	10	Masseters, orbicularis oris	0	Antidopaminergic, anticholinergic	Upper face, lower face, pharynx, neck	–
15	85 F	1	Masseters, temporalis, ext pterygoids	75	Anticholinergic, baclofen, clonazepam, antidopaminergic	Lower face, tongue	+
16	74 F	3	Masseters, temporalis	75	Anticholinergic, baclofen, clonazepam, carbamazepine, dopaminergic, antidopaminergic	Upper face, lower face, pharynx, neck	–
17	64 F	9	Masseters, temporalis	0-10	Anticholinergic, antidopaminergic, clonazepam, baclofen, dopaminergic	Upper face, lower face, pharynx, neck	–
18†	54 F	15	Masseters, ext pterygoids	0-10	Anticholinergic, antidopaminergic	Upper face, lower face, R arm, pharynx	–
19	68 F	11	Masseters	85	Dopaminergic, antidopaminergic	Upper face, lower face	–
20	53 F	2	Ext pterygoids	95	Baclofen, ethopropazine	Tongue	–

ext — external, int — internal.

*Anticholinergic — includes Artane and Parsidol; antidopaminergic — includes phenothiazines and other postsynaptic dopamine-blocking agents.

†Family history.

DISCUSSION

We and other investigators have used local injections of BOTOX for the treatment of blepharospasm,[15,16] torticollis,[13] spastic dysphonia,[14] and other regional dystonias.[13] We have found local injections of BOTOX valuable in relieving disabling dystonic spasms when limited to one muscle or group of muscles.

In most patients with OMD, related or unrelated to Meige's syndrome, dental appliances, psychotherapy, and physiotherapy have not significantly improved the jaw spasms, dysphagia, and dysarthria. Some authors have reported limited success with systemic pharmacotherapy. Drugs that enhance or decrease central neurotransmitter function produced a mild response in some patients with OMD.[9,10,17] Surgical removal of some of the masticatory muscles has been performed on some of these patients, with little benefit.

The BOTOX injections are performed on an ambulatory basis with little discomfort. The toxin is injected under EMG control for precise localization. Graded weakening can be achieved by using low doses initially, and then repeating the injections to achieve the optimum weakness desired, with a balance of muscular function. After titrating these doses, and with the muscles injected, one is able to determine the most appropriate dosage for future injections. If too much weakness is produced, the patient's strength gradually returns with time. It is too soon to know how often injections will be necessary.

Typically, the response of patients with OMD to drug therapy is poor, with a less than 25% improvement.[11] All of the patients were drug therapy failures before being invited to participate in our study. Therefore, an overall success rate of about 50% is a marked improvement over other available modalities. Of interest is that 11 (55%) had Meige's syndrome. We also found that five (25%) of the patients were treated previously for what was thought to be TMJ syndrome, with failure to control the symptoms with standard dental therapy in all cases.

The results of treatment found seven (35%) patients had a 0% to 10% improvement, one (5%) patient had a 10% to 20% improvement, three (15%) patients had a 50% improvement, and nine (45%) patients had a 75% or better improvement. The overall male response was 32%, whereas the women had a 50% improvement. The overall improvement of the entire group was 50%. We also found 45% of the patients to have a greater than 75% improvement.

Botulinum toxin is thought to affect the calcium disposal within the neurocytoplasm, thereby decreasing the concentration of calcium to subthreshold levels. This then prevents the release of acetylcholine into the synaptic gap.[18] As a result, BOTOX weakens muscles and prevents tight spasms and associated symptoms.

In contrast to systemic pharmacotherapy with cognitive and sedative side effects, local injections of BOTOX were without clinically significant systemic side effects. The long-term effects of BOTOX on the masticatory muscles are not yet known. So far we have not found any disability produced by the toxin.

Others have reported a subclinical defect of neuromuscular transmission seen on single fiber EMG of limb muscles distal to the site of injection in patients treated with much larger doses for torticollis.[19] Previously we[13] reported the production of antibodies to BOTOX A in patients treated with large doses for torticollis. These observations suggest spread into the bloodstream; there is a theoretic risk of developing additional immunologically mediated responses, such as anaphylaxis.

SUMMARY

Oromandibular dystonia is a disorder that affects the masticatory, lower facial, and tongue muscles, producing spasms and jaw deviation. The symptoms are usually related to eating, speaking, and pain. These symptoms may present alone, often simulating TMJ syndrome, or in association with other focal dystonic movements (as in Meige's syndrome), or as part of a more generalized dystonia.

We have used BOTOX to treat more than 239 patients with focal dystonias, including 20 with OMD. Botulinum toxin was injected directly into the masticatory muscles under EMG localization of the appropriate muscles. We achieved an overall response of about 50%, with several patients achieving an 85% to 95% improvement in eating, speaking, and/or pain control. In some of the patients the results were augmented with continued use of systemic medication.

Much work still remains in understanding the cause of dystonia and the action of BOTOX. The dosage of toxin, the frequency of injection, and the long-term and short-term effects on muscle are similarly uncertain. We feel, however, that BOTOX has an important role in the management of patients suffering from dystonias including OMD.

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ACUTE MANAGEMENT OF LARYNGEAL TRAUMA

UPDATE

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The management of 120 laryngeal injury patients at a major trauma center over 23 years is presented in the form of two patient populations. The first population consists of 52 previously analyzed and reported laryngeal trauma patients who served as the basis for the subsequent refinement of treatment of a second population of 68 patients. Collectively, these patients form a series unique in that 1) it comprises the largest number of such injuries treated at one institution, 2) the management principles remained the same throughout the entire 23 years, and 3) the majority of the patients were managed by one physician. The experience gained from these patients is examined in the hope of resolving prevailing controversies about the treatment of the acutely injured larynx.

KEY WORDS — laryngeal trauma.

INTRODUCTION

The management of the acutely injured larynx remains a diagnostic and therapeutic challenge. The decisions made upon arrival to the emergency department will determine both the survival of the patient and the eventual outcome of the airway and voice. All agree that maintenance of the airway is the first priority, but there is disagreement as to the best way to establish that airway.[1,2] Further disagreement exists on everything from the indications for surgery to the timing and type of procedure.[3-15]

Despite the many reports in the past two decades, the above controversies continue because external laryngeal trauma remains a relatively uncommon injury, with few individuals having extensive experience.[1-16] As a result, recommendations for the management of blunt or penetrating trauma tend to follow from retrospective reviews of a limited number of patients treated by different physicians at one institution or the incorporation of multiple previous reports into one article. In such a reporting system, differing opinions as to diagnosis and treatment are slow to be resolved, and indeed, one author has stated, "Each case presents a different problem, and it is difficult to follow a set routine on each one."[16] We believe that the infrastructure of cumulative experience now exists to offer sufficient insight into the management of such injuries. We also believe that such controversies are best resolved in the context of one large series of such patients managed in the same manner by the same individuals. As have others, we have been concerned over the past decade with the issues of 1) improving our diagnostic strategies for both blunt and penetrating laryngeal trauma, 2) assessing the risks of establishing an airway by intubation versus local tracheotomy, 3) refining the criteria for surgical management, 4) analyzing the benefit of immediate rather than delayed surgical treatment, 5) evaluating the role of primary closure versus skin grafts for the repair of severe mucosal lacerations, 6) identifying those patients who will require "lumen-keepers," 7) examining restoration of normal anatomy versus resection, and 8) establishing reasonable expectations following such injuries.[1,17,18] In this report, we have the unusual opportunity to examine these issues from the perspective of a major trauma center treating a large number of laryngeal injuries by the same methods over an extended period of time. Over this time period, the results of this management have been evaluated, reported, and used to refine the treatment of subsequent patients.[1,17]

MATERIALS

This report discusses two patient populations. The first population is composed of 52 patients treated for acute laryngeal trauma between 1965 and 1978 at The University of Texas Southwestern Medical Center Affiliated Hospitals (Parkland Memorial Hospital, Veterans Administration Medical Center, Presbyterian Hospital, and John Peter Smith Hospital). The treatment results of these patients previously have been analyzed retrospectively and formed the recommendations for the management of all subsequent patients.[17] The second population consists of 68 laryngeal trauma patients prospectively managed under the direct supervision of one of the authors (S.D.S.) over the past 10 years, primarily at Parkland Memorial Hospital (Table 1). Since this report encompasses a period of 23 years, patient information was derived either from records kept by one of the authors at the time of injury and/or from chart review. Follow-up ranged from 1 month to 9 years after injury in the first patient population and from less than 1 month to 5 years in the second population. With the exception of two patients treated within 36 hours, diagnosis and

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TABLE 1. TREATMENT GROUPS (N = 120)

Group	*Retrospective (1965-1977)*	*Prospective (1978-1988)*	*Total*
1	7	11	18
2	5	35	40
3	14	12	26
4	26*	10	36
			120

*Excludes one patient in previous report.[1]

treatment of all patients was performed within 24 hours of injury. Patients initially managed at other institutions were excluded from this report (one patient in the retrospective population was eliminated for this reason). This exclusion criterion was necessary to keep the theme of the report consistent; that is, the early management of acute laryngeal injuries, rather than secondary management of the late sequelae of laryngeal trauma. Although several series have included isolated tracheal injuries as part of a broader category of "laryngotracheal trauma," this report is confined solely to laryngeal injuries.[2]

MANAGEMENT

The diagnosis of laryngeal trauma was based on history and physical examination (Table 2). Emergent measures included preservation of the airway, cardiac resuscitation, control of hemorrhage, and stabilization of neural and spinal injuries followed by a systematic search for other organ system trauma. Preferred management of the unstable airway was by tracheotomy rather than endotracheal intubation. Our treatment protocol is represented diagrammatically in Fig 1. In the initial 17 years of the study period, soft tissue radiographic examination of the neck was performed routinely, along with cervical spine radiography. In the last 5 years, computed tomographic scanning has replaced soft tissue radiographs and has modified our treatment approach. We believe that CT scanning is most efficacious when the results influence the actual clinical course rather than document an obvious injury in which the treatment is unchanged by the radiographic findings.[18] Over the entire study period, management was divided into four groups according to the severity of the injury.

Group 1. Findings included minor endolaryngeal hematomas or lacerations, and absence of a detectable fracture of the laryngeal skeleton. Computed tomography was used to confirm the integrity of the laryngeal cartilages. Patients were managed by a minimum of 24 hours of close observation, head-of-bed elevation, and humidification of inspired air. Steroids were prescribed inconsistently both in this group and in group 2.

Group 2. Findings included edema, hematomas, or minor mucosal disruption without exposed cartilage. Patients were managed by initial tracheotomy to preserve the airway and allow the administration of general anesthesia without endotracheal intubation. This was followed by direct laryngoscopy and rigid esophagoscopy to diagnose the extent of injury. When at all possible, tracheotomy was performed with local anesthesia to avoid further disruption of the airway by the endotracheal tube. As was true of group 1, no further operative intervention was attempted because of the likelihood of spontaneous resolution of the injury. Beginning in years 16 and 17, CT was used initially to confirm laryngeal fractures in patients who were difficult to examine by either indirect or direct laryngoscopy because of massive endolaryngeal edema or hematomas. Prior to these years such patients might have undergone open exploration of the larynx to determine the extent of their injury. With subsequent experience, open exploration was considered unnecessary in this group if there were no fractures or if there were single fractures that were nondisplaced.

Groups 3 and 4. Findings included massive edema, large mucosal lacerations, exposed cartilage, displaced fractures, and vocal fold immobility. Following tracheotomy and endoscopy, the larynx was explored either through a midline thyrotomy or through a laryngofissure via the fracture site in the case of paramedian fractures. Computed tomography was omitted, because these injuries were so significant that it was apparent that restorative surgery was required. All mucosal injuries were identified and approximated carefully with 5-0 or 6-0 absorbable sutures. The anterior margin of each true vocal fold was sutured to the thyroid cartilage or its external perichondrium at the thyrotomy site to reconstitute the normal scaphoid configuration of the anterior commissure. Based on the experience in the first patient population, this technique proved useful in the second population in avoiding the use of stents.

Group 4 patients differed from group 3 in that their injuries tended to be more severe, leaving the

TABLE 2. PROSPECTIVE POPULATION PROFILE (N = 68)

	Presenting Symptoms							*Type of Injury*		
Group	*Hoarseness*	*Pain/ Tenderness*	*Hemoptysis*	*Dysphagia*	*Subcutaneous Emphysema*	*Impaired Respiration*	*Hematoma*	*MVA*	*Blunt*	*Penetrating*
1	8	7	1	1	1	1	0	7	3	1
2	10	3	2	2	5	18	3	7	9	19
3	4	4	1	1	5	8	1	2	2	8
4	3	3	0	1	2	6	0	2	2	6

MVA — motor vehicle accident.

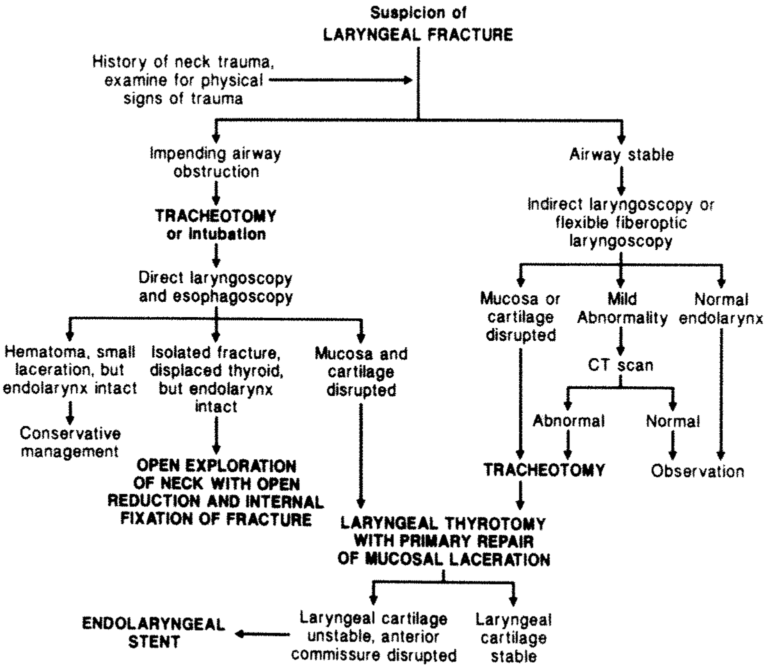
Suspicion of LARYNGEAL FRACTURE
History of neck trauma, examine for physical signs of trauma
Impending airway obstruction
TRACHEOTOMY or intubation
Direct laryngoscopy and esophagoscopy
Hematoma, small laceration, but endolarynx intact
Conservative management
Isolated fracture, displaced thyroid, but endolarynx intact
OPEN EXPLORATION OF NECK WITH OPEN REDUCTION AND INTERNAL FIXATION OF FRACTURE
Mucosa and cartilage disrupted
Airway stable
Indirect laryngoscopy or flexible fiberoptic laryngoscopy
Mucosa or cartilage disrupted
Mild Abnormality
Normal endolarynx
CT scan
Abnormal
Normal
TRACHEOTOMY
Observation
LARYNGEAL THYROTOMY WITH PRIMARY REPAIR OF MUCOSAL LACERATION
Laryngeal cartilage unstable, anterior commissure disrupted
Laryngeal cartilage stable
ENDOLARYNGEAL STENT

Fig 1. Diagrammatic representation of treatment protocol for laryngeal trauma.

anterior half of the larynx (true and/or false vocal fold) disrupted or the cartilaginous skeleton unstable. Therefore, to avoid adhesions in the anterior larynx or collapse or overriding of fragments of cartilage, a lumen-keeping stent was employed. In the retrospective population, stenting was performed with commercially available stents, a finger of a surgical glove filled with sponge rubber, or a Portex endotracheal tube stent. After 1975, the Portex stent was used exclusively. These "lumen-keepers" were fixated with 2-0 Prolene sutures passed externally through the laryngeal ventricle and cricothyroid membrane. Before this period stents were held in place by wire, which thereafter was abandoned because of breakage in three patients. The stent was prepared by cross clamping a 3- to 4-cm segment of a Portex endotracheal tube and autoclaving it to 82°C as has been described previously.[19] Stents were removed at direct laryngoscopy with general anesthesia at approximately 4 weeks after injury until 1983, when the authors realized that 2 weeks would suffice to stabilize the larynx and avoid the complications of further stenting. At no time during the 23 years of this study were skin or mucous membrane grafts used. Fractured cartilage was repaired with either wire or nonabsorbable suture material, with the latter most often used in the prospective group. Removal or debridement of the laryngeal skeleton was limited to fragments that were avulsed totally from their perichondrial blood supply. Despite the severity of many of the injuries, the goal of the operations remained restoration of normal anatomic relationships in the hope of best preserving airway and vocal function.

RESULTS

The findings in this study are reported in terms of incidence of laryngeal trauma and mechanism of injury over time, initial airway management, presenting symptoms, diagnostic improvements, restoration of airway and voice, and complications.

Incidence and Mechanism of Injury. As previously stated, patients were treated primarily at Parkland Memorial Hospital. This hospital is the level 1 trauma center for Dallas County, treating 158,874 emergency patients in 1987. The large population reflects the special character of Parkland as a trauma center and the close relationship between the hospital and the community. Given these circumstances, it is understandable why many large series of blunt and penetrating trauma have originated from this hospital.[20] During the first 13 years of this study, 48 patients were identified at Parkland as having sustained either acute blunt or penetrating trauma. During this period, emergency room visits average 155,780 per year. In the subsequent 10 years, 68 patients were seen with this problem while the emergency room saw approximately 157,000 patients per year. These figures would imply that the actual incidence of this injury is increasing. We believe this is not the case, but rather reflects our better ability to identify these patients and the close supervision of their care by one of the authors. In comparing these two populations there is an obvious shift from blunt to penetrating trauma as the mechanism of injury. This reflects both 1) reduction in the speed limit and, more recently, the introduction of a mandatory seat belt usage law and 2) the overall increase in personal assaults in Dallas County.

Initial Airway Management. Prior to our first report covering the treatment of laryngeal injuries at The University of Texas Southwestern Medical

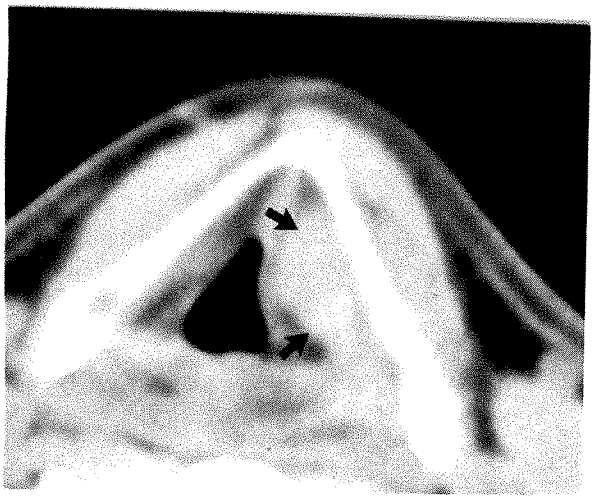

Fig 2. Axial computed tomogram showing edema and hematoma (arrows) involving left false vocal fold. Laryngeal skeleton is intact, without evidence of fracture. Patient was 22-year-old man who presented with stridor and hoarseness after striking neck on dashboard of automobile. Patient made full recovery after initial tracheotomy for airway management followed by endoscopy.

Center, there was no absolute agreement between the emergency room personnel and the trauma and otolaryngology services on the best way to establish an airway. This first report revealed four patients who lost their airway during attempted orotracheal intubation, with successful resuscitation via an emergency airway.[17] Thereafter, the "preferred" management of the airway has been by tracheotomy under local anesthesia. Unfortunately, some injuries are not recognized immediately or the circumstances do not lend themselves toward an emergency tracheotomy. The subsequent effect of orotracheal intubation has varied from no apparent adverse outcome to further disruption of the larynx by the endotracheal tube and even to temporary loss of the airway.

Presenting Symptoms. The presenting symptoms and method of injury for the prospective population by management group are summarized in Table 2. The only valid inference from this data is that the worse the injury, the more likely the patient is to have difficulty breathing. The rest of the "classic" signs and symptoms of laryngeal trauma probably are not noted with increasing severity of injury because of the need to establish an immediate airway.

Diagnostic Improvements. As previously stated, CT scanning has been employed selectively in the last 5 years to decide whether exploration of the larynx is indicated.[18] Using the criterion of multiple or displaced fractures as an indication for open exploration, several patients with only massive endolaryngeal edema have been treated successfully without thyrotomy (Fig 2). Others have had questionable findings on physical examinations and CT has clearly identified displaced fractures.

Restoration of Airway and Voice. The results by management group for both the retrospectively and prospectively treated patients are summarized in Table 3. For consistency, we have employed the same measures of success as used in our previous reports.[1,17] A good voice was one that was close to normal or comparable to the patient's preinjury voice. Moderate to marked hoarseness was described as a fair voice, and phonation just above a whisper was classified as poor. Airway quality was judged good if the patient denied restriction, fair if mild to moderate restriction persisted, and poor if permanent tracheotomy was necessary. It should be noted that despite the severity of several of the blunt and gunshot injuries, no patient was treated by partial or total laryngectomy.

In group 1, all 18 patients (seven retrospective and 11 prospective) obtained a good airway and a good voice. In group 2, 32 of 40 (five retrospective and 32 prospective) patients were considered evaluable. Two of the nonevaluable patients died of their injuries, one from a concurrent crushed chest and the other from a gunshot wound to the cervical spine. The third nonevaluable patient achieved a good voice, but never returned for removal of his tracheotomy tube. Five other patients did not return for follow-up care. The results of the 32 evaluable prospective patients are summarized in Table 3. The two with fair voice results reflect a recurrent laryngeal nerve injury in one patient and an unrepaired laceration of the anterior third of the true vocal fold in the other patient. Thirteen prospectively managed patients in this group underwent concurrent open neck exploration without thyrotomy for vascular, pharyngeal, and esophageal injuries. Three of these patients had minor fracture dislocations of the thyroid cartilage, which were reduced and fixated without entry into the larynx. One patient had a prolonged hospitalization due to a pharyngocutaneous fistula following a gunshot wound. The other complication in this group was temporary loss of the airway due to attempted orotracheal intubation in a patient with an initially unrecognized laryngeal injury. Follow-up in both groups 1 and 2 tended to be short, because patients often did not return once they were well and/or decannulated (range, 2 weeks to 5 years).

In group 3, 19 of the 26 patients (eight retrospective and 11 prospective) were available for follow-up ranging from 1 month to 5 years. Reasons for nonevaluability ranged from death due to complications from other injuries to failure to return after removal of the tracheotomy tube. All evaluable patients were decannulated in 5 to 35 days. Two patients had significant fracture dislocations of the thyroid cartilage without endolaryngeal lacerations and were managed successfully by reduction of the fracture and approximation of the external perichondrium and thyroid cartilage without entry into

TABLE 3. RESULTS OF TREATMENT OF EVALUABLE PATIENTS (N = 99)

	Voice			*Airway*		
Group	*Good*	*Fair*	*Poor*	*Good*	*Fair*	*Poor*
1	18	0	0	18	0	0
2	30	2	0	31	1	0
3	16*	3	0	19	0	0
4†	20	9	0	28	0	2

*One patient achieved good voice after Teflon injection of true vocal cord following recurrent nerve injury.

†One patient not evaluated for voice because of prolonged coma. Another patient required removal of polyp and then developed fair voice.

the larynx. Six months after injury, one patient in the prospective group underwent left true vocal fold Teflon injection for recurrent nerve paralysis. Following this procedure the patient achieved a good voice.

Thirty of the 36 group 4 patients (20 retrospective and ten prospective) were evaluable, with follow-up ranging from 2 months to 10 years postinjury. It is interesting to note, and certainly tragic, that two patients were not evaluable for such reasons as death by assault 2 days after removal of the laryngeal stent. Decannulation varied from 27 to 100 days after injury, with two patients in the retrospective group requiring permanent tracheotomy. In one of these patients, cannulation was required for pulmonary toilet necessary for anoxic brain damage following cardiac arrest. The other patient was an elderly woman who continued to use a small-diameter tracheotomy tube with a fair voice and a fair airway clinically (classified as a poor airway in this study because of the persistent tracheotomy) but refused decannulation. Voice and airway results are summarized in Table 3. Complications in group 4 patients included 1) avulsion of the mucous membrane of the hemilarynx from attempted intubation in one patient, 2) slippage or expulsion of the stent secondary to wire breakage in three retrospective patients, 3) total airway obstruction following attempted endotracheal intubation in four patients who were rescued by tracheotomy, and 4) removal of an anterior commissure polyp resulting in a fair voice in one patient. Another patient, previously reported as developing perichondritis 9 years after injury, was later found to have carcinoma of the larynx.[1]

Antibiotics, primarily cephalosporins in the prospective group, were given for 5 to 10 days after injury in group 3 and 4 patients. Steroids were not used in these two groups of patients, but were employed inconsistently in the other two groups.

DISCUSSION

The results of 23 years of management of acute laryngeal trauma at one institution, and primarily by one surgeon, are reported. The 120 patients in this series were divided into retrospective and prospective management populations. The majority of the patients in the retrospective group were not treated by either of the authors, but rather represented a source of information that allowed for the refinement of the management principles that were employed in the prospective population. Collectively, both populations form a uniquely large laryngeal trauma series that may serve to resolve some of the remaining controversies in the management of this injury.

Acute Management of Airway. We have previously stated, "There is an unfortunate tendency for nonlaryngologists to regard all airway emergencies as best managed by endotracheal intubation."[1] We continue to believe that intubation following laryngeal trauma is hazardous. These statements are contrary to the recommendations of other authors, such as Gussack et al,[2] who advocate orotracheal intubation over tracheotomy with local anesthesia. Others share this opinion, reporting various levels of success.[21,22] We would suggest that the real issue is not how many patients have not had an adverse outcome from orotracheal intubation, but rather the total avoidance of either loss of or damage to the airway. We do believe that the advent of flexible fiberoptic laryngoscopy and CT scanning allows for the evaluation of the larynx in those patients with minimal trauma (eg, slight edema, small hematomas, or small lacerations). Such patients who require a general anesthetic for treatment of other injuries can undergo careful intubation by an experienced physician. Otherwise, our experience suggests that the routine management in laryngeal trauma of the airway by intubation remains hazardous.

Changing Diagnostic Strategies. Although sometimes used interchangeably, documentation and diagnosis of laryngeal trauma are separate indications for radiography in these injuries. We feel that radiography should be reserved for establishing the severity of injury so that treatment follows new or vital information gained from this examination. We believe that either multiple or displaced fractures of the thyroid or cricoid cartilages require open reduction and fixation by sutures placed through the external perichondrium or cartilage. In the past we have not recommended such treatment in single, nondisplaced fractures. The recent work of Stanley et al[23] on excised larynges suggests that these injuries, too, should be fixated to avoid changes in the voice. We continue to question the use of CT in injuries in which findings of the physical examination indicate an obvious need for reduction and fixation (ie, the crushed larynx). In addition, we feel strongly that the use of laryngograms is contraindicated, and even dangerous, in these unstable airways, despite their recommendation by previous authors.[8] The advent of flexible fiberoptic laryngoscopy proved in the last 5 years of this series to be a useful diagnostic modality. We have found it to be superior to indirect laryngoscopy, both in quality of information gained and patient cooperation. This procedure is used best as an alternative to rigid direct laryngoscopy in patients suspected of having minimal injuries. Esophagoscopy remains a useful adjuvant procedure in patients undergoing direct laryngoscopy.[9] In this series, several patients were found at esophagoscopy to have unsuspected esophageal perforations.

Operative Intervention. We believe that beyond the need to establish an airway by tracheotomy or evaluate the injury by direct laryngoscopy, surgical therapy should be considered when spontaneous resolution of the laryngeal injury is unlikely.[1] The results of treatment in those patients in groups 1 and 2 suggest that a full recovery without further operative intervention is likely with laryngeal edema, hematomas without mucosal disruption, small lacerations of the endolarynx not involving the anterior commissure, and small lacerations of the supraglottic larynx that do not disrupt the integrity of this site. Single, nondisplaced thyroid cartilage fractures appeared to do well with only medical therapy. However, we have been using subjective criteria to judge voice results (eg, the patient's opinion of his or her voice), and it is possible that the changes in vocal dynamics (eg, glottal resistance, signal amplitude, and noise content) reported by Stanley et al[23] are not detected by our simplistic methods. The only clinical experience confirming the efficacy of such intensified management consists of a report by Hirano et al[24] that demonstrated return of high-pitched phonation following reduction of a paramedian thyroid fracture. We support these authors' recommendation for more intensive evaluation and documentation by vocal function and electromyographic testing of what we previously have considered "lesser injuries" in the hope of refining the criteria for surgery. Until more is understood about the significance of these lesser injuries, we feel that as a minimum all displaced, or single nondisplaced, potentially unstable fractures probably are best reduced and fixated.

Open Versus Closed Reduction. Throughout this series the method of reduction of fractures remained open exploration of the injury and fixation by suture or wire. We continue to recommend this form of management over closed reduction. We believe closed reduction is difficult and that the stresses on the fracture due to the dynamic nature of the larynx require fixation. We have found that a single displaced fracture, such as inward collapse of one thyroid lamina, can be repositioned and fixated without a laryngofissure. With more severe injuries, open exploration via a laryngofissure or thyrotomy clearly is indicated. Such injuries include large mucosal lacerations (particularly of the anterior commissure), displaced or comminuted cartilage fractures, avulsed mucosa, or dislocated arytenoids.

Conservation of Anatomy. Despite the recommendations of some authors, skin grafts were not required to cover exposed wounds in any of the 62 group 3 and 4 patients, because all injuries were closed by primary approximation of laryngeal mucous membrane.[3] Other authors have reported their experience in managing major laryngeal injuries following gunshot wounds by total laryngectomy.[25] Still other authors recommend resection of the comminuted cricoid.[4] Although we have seen many such injuries, the results of this series support the concept of restoration of normal anatomy by careful closure of mucous membrane and fixation of fractures. At times this has resulted in 1) a foreshortening of the anterior-posterior dimension of the larynx due to loss of the anterior thyroid cartilage, 2) use of muscle flaps to replace a missing anterior cricoid ring, or 3) mucosal advancement to relieve tension on suture lines.

Blunt Versus Penetrating Trauma. Over the past 30 years, there have been various discussions as to the significance of blunt versus penetrating trauma in the patient outcome. In this series, the greater the actual trauma, the poorer the results. There was no apparent difference in voice or airway results between these two groups.

Operative Timing. Early versus delayed operative management of acute laryngeal trauma remains somewhat controversial. Some authors have recommended waiting from 3 to 5 days after injury to permit edema to resolve.[3,5,13] They reason that waiting allows better identification and approximation of endolaryngeal lacerations. Others have advocated closure of such wounds as soon as possible.[1,12,14] Although we believe the variations in treatment and reporting between other laryngeal trauma studies make statistical comparisons impossible, the results of the present series are sufficiently good, and the population of significant enough size, to attest to the validity of immediate operative treatment. We feel that the early operative intervention permitted primary closure of the injured larynx, and we question the wisdom of suggesting the intentional delay of treating these wounds, just as we would question the policy of electively waiting to close facial wounds several days after injury. Since all patients were managed in our series within 24 hours, no comparison can be made with an alternatively treated population.

Indications for Stenting. In attempting to understand which patient will benefit from stenting, the physician must ask what is to be gained by the use of a stent or lumen-keeper. In the acute injury, the stent should serve as an internal fixation device. The stent also should prevent scarring between endolaryngeal lacerations and maintain the internal configuration of the larynx. While several clinical studies and our own experience showed no significant problems with short-term stenting, experimental laryngeal injuries in dogs have demonstrated an increased incidence of infection and granulation tissue with a variety of "soft stents."[26] These considerations led us to employ a molded Portex stent over the past 10 years only when 1) numerous cartilaginous fractures precluded stabilizing the laryngeal skeleton by open reduction and internal fixation alone or 2) extensive lacerations or avulsion injury prevented restoration of a normal anterior commissure because of probable web formation or blunting of this site. With careful closure of all wounds, our results imply sufficient stability of both fractures and mucous membrane wounds to permit removal of stents at 2 weeks.

Expectations. In recent years, there is an evident trend in the literature toward improved outcome following laryngeal trauma. In part, this trend reflects the early recognition of these injuries and at least some consensus on management principles. In the present report, an excellent recovery was observed with or without minimal surgical intervention (eg, tracheotomy and endoscopy) in patients with edema, hematomas, or small lacerations. Early repair and stabilization of severe lacerations and fractures, with selective use of stents, yielded an overall good return of both airway and vocal function. We hope that our experience will serve to guide others in the management of these infrequent and demanding injuries.

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LASER SURGERY FOR VOCAL CORD CARCINOMA INVOLVING THE ANTERIOR COMMISSURE

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Endoscopic laser surgery is an established means of treatment for benign laryngeal lesions. Laser surgery for early (stages I and II) squamous cell carcinoma is still being tested. Treatment of glottic tumors extending to the anterior commissure is in itself controversial. Approximately 20% of all glottic tumors involve the anterior commissure, with only 1% of these lesions being purely anterior commissure tumors. The anatomy of the anterior commissure is such that an apparent T1 lesion may actually be a T4 lesion if it involves the thyroid cartilage. The distance between the anterior commissure ligament and the thyroid cartilage is only 2 to 3 mm. A preoperative computed tomographic scan can aid us in evaluating this space. Therefore, tumors of the anterior commissure present as a therapeutic challenge. Radiation therapy has proven to be inadequate, with a high rate of recurrence and increased risk for radiochondronecrosis. The literature with regard to radiotherapy varies widely as to survival rates. Conservation surgery has consistently demonstrated an 80% survival in T1 lesions. Recently, it has been suggested that laser surgery in the region of the anterior commissure might offer satisfactory results. We have found the opposite. We will report on five patients who underwent endoscopic laser surgery on T1 vocal cord lesions involving the anterior commissure. All of these patients had tumor recurrence and subsequently have undergone salvage surgery and/or radiation therapy. The difficulties associated with endoscopic laser surgery of the anterior commissure will be discussed with a supporting animal study.

KEY WORDS — anterior commissure, glottic tumors, laryngeal commissure, laser surgery.

INTRODUCTION

Management of glottic tumors involving the anterior commissure remains controversial. Early T1 or T2 squamous cell carcinomas of the anterior commissure can be treated with a variety of therapeutic modalities. The treatment of choice is determined by the surgeon's philosophy of how these tumors might invade the surrounding tissues, the quality of voice achieved after treatment, the risks and complications of the treatment chosen, and the long-term survival.

Conservation surgery of the larynx has grown and been refined since Clerf[1] first reported treatment of vocal cord cancer by laryngofissure in 1940. For tumors involving the anterior commissure, he recommended removing an anterior triangle of thyroid cartilage. Leroux-Robert in 1956[2] discussed the partial frontoanterior and partial frontolateral laryngectomies that opened an era of conservation surgery for vocal cord cancer. Norris[3] introduced the extended frontolateral laryngectomy in 1958, and Som and Silver[4] described the anterior commissure technique of vertical partial laryngectomy in 1968 that is the basic procedure still used today.

Endoscopic management of laryngeal tumors is not a new concept. In 1920, Lynch[5] reported on 39 patients with early glottic cancers who were successfully treated with transoral excision with a 90% cure rate. New and Dorton[6] in 1941 reported their success with transoral excision and diathermy (Bovie electrocautery). Eleven patients were treated for intralaryngeal lesions described as small tumors involving the anterior two thirds of the vocal cord without fixation; follow-up indicated a 90% cure rate. In 1973, Lillie and DeSanto[7] reported on 98 patients with T1 glottic carcinoma not involving the anterior commissure who were treated with transoral excision; all were cured, although five required further treatment. In the analysis of these patients, it is not possible to determine what percentage of cases involved the anterior commissure.

Radiation therapy for laryngeal carcinoma can be traced back to 1920 and the work of Coutard.[8] In the early years of external radiation therapy, the limiting factors of total dosage and protraction (early form of fractionation) were based on mucosal and skin reactions to therapy. The period from 1930 to 1950 was one of wide application of radiation therapy, with use of more powerful machines that reduced the length of early treatment.[9] By 1951, cobalt 60 units were being used clinically. At this time, therapists were beginning to limit their total therapy to the equivalent of the currently accepted 6,000 rad in fractionated doses. The value of radiation therapy during the developmental years is difficult to compare to today's standards because of the different classifications of these endolaryngeal tumors. Today, the results obtained with radiation therapy for T1 lesions of the larynx have been similar to those obtained surgically.[10,11]

Laser surgery naturally evolved as an extension of endoscopic surgery in 1972 with the work of Jako[12] in the animal laboratory and with Strong et al[13] in

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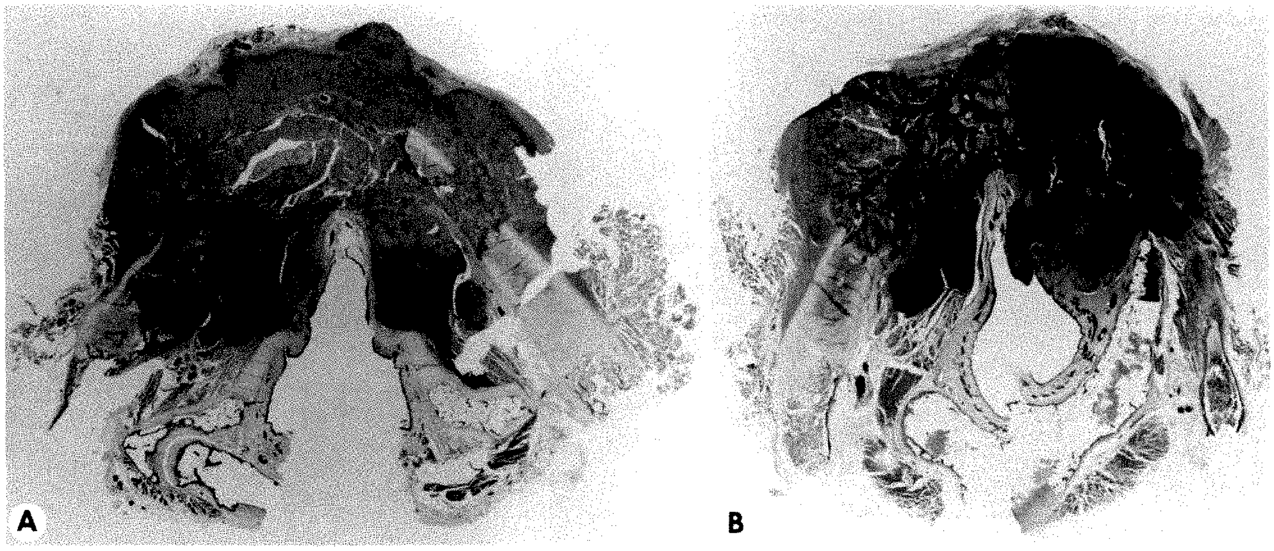

Fig 1. (Case 1) Photomicrographs of histopathologic findings from whole organ serial sections of larynx. A) Recurrent disease has invaded through anterior commissure. B) Deeper cut demonstrates tumor replacing anterior thyroid cartilage.

1973, when they reported their results on 142 procedures. The role and limitations of laser surgery in the treatment of glottic tumors still is being defined. Favorable reports on use of laser surgery on early T1 malignancies of the vocal cords not involving the anterior commissure have been described in the literature.[14]

The anterior commissure is anatomically different from the rest of the larynx. Tumors of the anterior commissure may not impair vocal cord mobility because of the lack of invasion of the vocalis muscle. However, since only 2 to 3 mm separates the anterior commissure mucosa from the thyroid cartilage, a small tumor on the surface actually may invade the cartilage, thus changing the staging from T1 to T4. These factors greatly contribute to the controversy in the management of these tumors.

MATERIALS AND METHODS

Five patients with stage I vocal cord tumors involving the anterior commissure are presented. They were treated between 1984 and 1986. All were men from 62 to 90 years of age who had direct laryngoscopy performed, with a biopsy specimen sent for permanent section as part of their treatment planning. In addition, all had computed tomographic scan evaluation to rule out thyroid cartilage invasion. Well-differentiated squamous cell carcinoma without invasion of the thyroid cartilage was found in each of the patients.

All of the patients chose laser surgery over other treatment options after discussion of the biopsy and scan results. The procedure was done with general anesthetic introduced with a Rusch red rubber endotracheal tube wrapped with aluminum tape. Suspension microlaryngoscopy was performed with an Ossoff-Karlan laser laryngoscope (Pilling, Fort Washington, Pa) and Lewy suspension apparatus (Storz, St Louis). The advantages of this laryngoscope, in addition to its wide aperture, are the separate smoke-evacuator channel and double light source channels. Standard laser safety techniques were employed.

The operating microscope with a 400-mm lens was attached to the carbon dioxide laser. With use of repeat and continuous modes between 10 and 14 W, the tumor was excised. Biopsy specimens of the tumor margins (superficial and deep) were sent for frozen section. The patients tolerated the procedure well.

CASE REPORTS

Case 1. A 90-year-old man presented with a history of progressive hoarseness without dyspnea or dysphagia. He had not smoked for 23 years prior to presentation. He underwent direct laryngoscopy and biopsy of a T1N0M0 right true vocal cord lesion with extension to the anterior commissure. A biopsy revealed squamous cell carcinoma. He had laser excision of the lesion. Results of a biopsy of an anterior vocalis muscle following the excision were negative. The patient did well for 11 months, until he returned with hemoptysis. Indirect laryngoscopy demonstrated a right transglottic lesion, and a total laryngectomy was performed without complications. Unfortunately, the patient died on the sixth postoperative day from a massive pulmonary embolism (Fig 1).

Case 2. A 76-year-old man was admitted with a 3- to 4-month history of progressive weight loss, poor appetite, and hoarseness. The patient denied dysphagia or dyspnea. His weight loss was attributed to chronic cholecystitis. Direct laryngoscopy revealed a horseshoe lesion of the anterior commis-

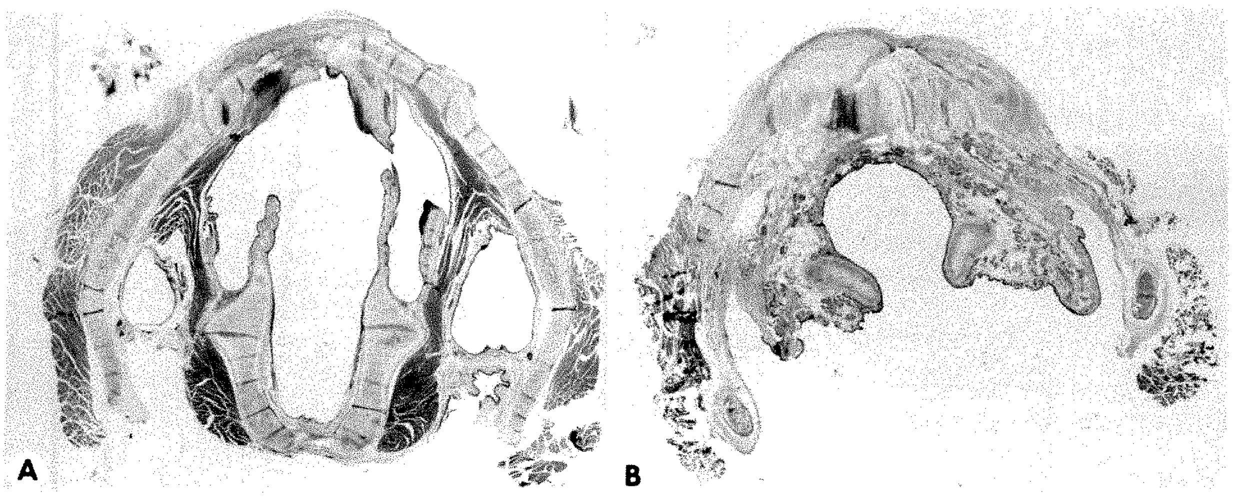

Fig 2. Photomicrographs of histopathologic findings from whole organ sections of larynx from mongrel dog. A) Viable tissue at anterior commissure, with intact anterior vocalis muscle, inner perichondrium, and cartilage, is noted after laser ablation. B) Deeper cut demonstrates inadequate ablation of anterior commissure with CO_2 laser.

sure. A biopsy frozen section demonstrated squamous cell carcinoma, which was totally excised with the CO_2 laser. The patient did well until approximately 1 year later, when he returned with recurrent disease. Total laryngectomy was performed. He is presently free of disease.

Case 3. A 59-year-old man presented with a 3- to 4-month history of hoarseness. He denied dyspnea, dysphagia, or odynophagia. He had smoked one pack of cigarettes per day for 40 years. The patient was found to have a T1 lesion of the left anterior vocal cord extending to the anterior commissure. He underwent direct laryngoscopy, biopsy, and laser excision of the tumor. Biopsy results following cordectomy were negative for tumor. He had a small recurrence of the left anterior true vocal cord lesion, which was lased 5 months later. After 10 months, he had repeat laryngoscopy and biopsy. Carcinoma again was found at the anterior commissure and the anterior right vocal cord. Partial laryngectomy was performed and he is presently free of tumor 2 years after surgery.

Case 4. A 79-year-old man presented with a 1-month history of hoarseness and odynophagia. The patient had been a heavy smoker and drinker. Direct laryngoscopy and biopsy results demonstrated a T1 squamous cell carcinoma of the right true vocal cord with extension to the anterior commissure. He underwent microlaryngoscopy with laser excision of the tumor and did well until 1 year later, when he had a repeat laser excision of a small right vocal cord recurrent lesion. Eight months later, he presented again with another superficial recurrent lesion at the anterior commissure that was treated with laser surgery. Six months after endoscopic excision he shows no evidence of disease.

Case 5. This 65-year-old man presented with hoarseness. Fiberoptic examination revealed a small friable lesion of the anterior commissure and the right true vocal cord. The patient had not smoked cigarettes or abused alcohol. Direct laryngoscopy and biopsy demonstrated a T1 squamous cell carcinoma. Laser cordectomy and excision of the anterior commissure was performed. Four months later, he was noted to have a recurrent lesion in the anterior commissure. At this time the patient opted for radiation therapy and completed 6,000 rad. Six months later he presented with another recurrent lesion of the anterior commissure. Wide-field total laryngectomy and superior mediastinal node dissection were performed. The biopsy specimen had negative margins and the mediastinal nodes were free of tumor. One year later he returned with an anterior pharyngeal mass. Partial pharyngectomy was performed with the creation of a controlled pharyngostome. Presently, he is without evidence of gross disease.

RESULTS

All patients initially did well without any postoperative complications. All five patients returned with recurrent disease from 3 months to 1 year following laser excision. The case 1 patient died postoperatively from a pulmonary embolism. The case 2, 3, 4, and 5 patients are presently free of tumor after surgery.

ANIMAL STUDY

We reevaluated our operative technique for laser excision of anterior vocal cords and commissure in the animal laboratory. The dogs were anesthetized intravenously with phenobarbital, which allowed spontaneous respiration without intubation. Direct laryngoscopy was performed with the Ossoff-Karlan laryngoscope and suspended with the Lewy suspension device. The CO_2 laser coupled to a microscope with a 400-mm lens was positioned. We then proceeded to lase the anterior vocal cords and commissure at 10 to 14 W repeat mode, with the attempt being made to totally excise all soft tissue to the cartilage. The dogs tolerated the procedure well and had no postoperative complications.

Three weeks later, the dogs were killed painlessly and total laryngectomy was performed. The larynx was preserved in 10% formalin and was sent for whole organ sectioning (Fig 2). Viable tissue was seen at the anterior commissure, with intact anterior vocalis muscle, inner perichondrium, and cartilage noted.

DISCUSSION

In recent literature the use of laser surgery for early vocal cord tumors has been praised.[15,16] Our results would discourage the use of the CO_2 laser in the treatment of early lesions involving the anterior commissure.

The anterior commissure of the larynx as defined by Sessions et al[17] is limited by the upper surface of the true vocal cords superiorly, the lower surface of the true vocal cords to 5 mm subglottically, the thyroid ala at the insertion of the anterior commissure tendon anteriorly, and the free margins of the true vocal cords to 2 mm on each side posteriorly. The anterior commissure tendon, or Broyles's ligament, extends from the upper portion of the thyroid cartilage caudad for approximately 10 mm and is about 1 mm in width.[18] Olofsson et al[19] extended this definition to include the thyroepiglottic petiole. Bagatella and Bignardi[20] have described further the limits of the anterior commissure with what they have designated the "x-space," which separates the anterior border of the supraglottis from the glottis. The x-space is composed of dense connective tissue at the upper side of the true vocal cord and is morphologically different from the vascular and glandular network that makes up Broyles's ligament. The x-space seems to act as a barrier against spread of tumors from the supraglottic region to the glottic region. As Tucker et al[21] emphasize, a thorough understanding of how tumor spreads from the anterior commissure is the key to deciding the therapeutic intervention to be taken.

Anterior commissure tumor invasion of the thyroid cartilage is attributed to lack of perichondrium where the anterior commissure tendons insert into the thyroid cartilage.[19] Perichondrium acts as a strong barrier to tumor spread.[22] Yeager and Archer[23] found malignant cells growing along the collagen bundles that make up the anterior commissure tendon, thus forming linear passageways through which they invade the cartilage. Therefore, with the anatomic limits of the anterior commissure reasonably defined and methods of tumor invasion demonstrated, the treatment chosen needs to take into account the possible invasiveness of the tumor.

The anterior commissure is a space whose borders have been described as a result of experience with treatment of tumors in this area, but is otherwise not a true space with physical limitations. The anterior and superior borders of the anterior commissure are well demarcated, but inferiorly into the subglottic region and laterally along the true vocal cords the borders remain somewhat ambiguous. Experience with these tumors and their behavior has defined the limits of conservation surgery of the larynx. Tumors extending beyond these limits are classified and treated differently.[24]

The anterior commissure technique of partial vertical laryngectomy as described by Som and Silver[4] is oncologically sound for T1 and T2 glottic tumors involving the anterior commissure, with survival rates of 68% to 80%. Kirchner and Som,[25] using this technique, reported that almost 50% of their failures (8/18) were from local recurrence. Sessions et al[17] stated in their extensive review of 586 patients with glottic carcinoma that 180 patients had tumors involving the anterior commissure.

Radiation therapy as primary treatment for these tumors involving the anterior commissure also provides excellent results, with up to 80% 5-year survival reported for T2N0M0 lesions.[19] These results are as good as those obtained with conservation surgery, and those patients for whom radiotherapy fails may be offered salvage total laryngectomy, with limited success reported. Theoretically, radiotherapy treats the entire region involved, and therefore, it is not clear why treatment of any of these early lesions should fail. Perhaps these lesions are more extensive than originally staged and are not the early small lesions they were thought to be. The failure to eradicate these tumors despite high doses of radiation may be secondary to the technique used or the radiosensitivity of the tumor cells based on their cell cycle and tissue hypoxia.[26]

The CO_2 surgical laser is an adjuvant tool for endoscopic surgery of the larynx that offers excellent results in early carefully selected T1 carcinomas of the glottis.[27] The advantages and limitations of laser have been discussed by others.[15,16,27,28] Tissue removal is precise, healing is rapid, and hemostasis is good. Strong[27] emphasized in his early work with laser surgery of the larynx that "inadequate exposure and extension of the carcinoma to the anterior commissure, arytenoid, or subglottic area are contraindications to this approach." Koufman[15] reported excellent results with his experience on T1 glottic lesions; seven of his 16 patients had anterior commissure involvement and, except one who died of lung cancer, were tumor free 38 to 66 months later. Our experience with the anterior commissure has been to the contrary; all five of our patients required salvage surgery.

Whereas Davis and his coworkers[16] demonstrated virtually no anatomic limitations to endoscopic laser surgery of the larynx except the cricothyroid membrane anteriorly, they also noted that their work represented an ideal situation, with exposure of their isolated cadaver larynx not necessarily found in the clinical situation. In our in vivo study with dogs and even with good visualization of their larynges, we were still unable to excise completely the anterior commissure with the CO_2 laser; this was confirmed by histopathologic study. We feel that in our animal study there was as good if not better exposure of the canine larynx than found in vivo in the human larynx, and therefore, technique was not a factor in the results we obtained. In the human larynx, mechanical factors such as full dentition, a small oral cavity, and cervical spine disease may have limited the exposure of the anterior commissure, but in all cases, the larynx was suspended and the anterior commissure was thought to be adequately visualized.

We believe the problem with tumors located at the anterior commissure is their invasiveness combined with the lack of well-defined anatomic barriers against tumor spread. The microscopic spread of these tumors cannot be controlled adequately with the laser. In our hands, simply vaporizing the anterior commissure with the laser was not oncologically sound. The three-dimensional anatomy of the anterior commissure is too complex for the surgeon to excise a tumor thoroughly with adequate margins. Lesions confined to the middle of the vocal cords have borders usually better defined and, therefore, are more amenable to laser surgery. All five of our patients have required one or more salvage surgical procedures. Perhaps, if these patients had been treated with the conventional anterior commissure technique of partial vertical laryngectomy initially, then they would have been spared the increased morbidity of salvage procedures for recurrent disease. Today, we are no longer opting to use the laser for treatment of vocal cord tumors approaching or involving the anterior commissure.

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CHOANAL ATRESIA: TREATMENT TRENDS IN 47 PATIENTS OVER 33 YEARS

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From 1977 through 1985, 11 patients underwent surgical correction of choanal atresia. This group brings the total number of patients corrected at our institution since 1951 to 47. This review compares treatment and outcome in the most recent group with the previous series of 36 patients. Eight patients were female and three were male; their ages at the time of surgical treatment ranged from 1 day to 19 years. The atresia was bilateral in five patients and unilateral in six. Six patients had other physical or mental developmental problems. Mean follow-up was 5.6 years (range, 2 to 10 years). All five patients who had transnasal puncture as primary treatment required reoperation; this is a higher incidence of failure than in the previous group. None of the three patients who had transpalatal procedures needed further operation. In two of the eight patients who had transseptal repair, restenosis developed.

KEY WORDS — choanal atresia, surgical correction of choanal atresia.

From 1977 through 1985, 11 patients underwent surgical correction of choanal atresia at the Mayo Clinic. Eight were female and three were male; their average age was 8 years (range, 1 day to 19 years). The atresia was bilateral in five and unilateral in six patients. The occlusion was entirely bony in six and bony and membranous in five: no patients had purely membranous atresias. Six patients had other physical or mental developmental problems (Table 1). Follow-up was 100% (mean, 5.6 years; range, 2 to 10 years).

Five patients had transnasal puncture as primary treatment. Four of these patients had bilateral atresia; one had unilateral atresia. All were less than 3 months old at the time of first treatment and all required reoperation for restenosis. One patient had three punctures, three had two, and one had one. Four had eventual transseptal correction; one had a transpalatal approach. Two of the transseptal repairs restenosed and have not been reoperated. One patient who underwent transseptal correction had a puncture failure in our previous series.

Four patients had initial transseptal repair by a technique reported in our previous series.[1] Their average age was 7 years (range, 5 to 9 years). One patient had soft tissue restenosis that was excised and dilated, and the repair has remained patent. The other three repairs have remained patent.

Two patients had primary transpalatal repair at 4 months and 6 years. Both repairs are patent at follow-up of 3 years and 4.6 years, respectively. One patient had transpalatal repair at age 3 years, after two puncture failures, and the repair is patent at 5.4 years' follow-up.

Overall, the failure rate for transnasal puncture was 100%. Three of the five patients were treated at our institution and two were treated initially at other institutions. None of the transpalatal repairs failed and four of eight transseptal repairs restenosed; two remained open after minor revision and two have not been reoperated. All data are summarized in Table 2.

Many of the patients who had transseptal repairs in this series were younger than 8 years, whereas all of those in the previous series were older than 8 years and many were adults. In this study, as previously, age at time of surgical correction was correlated highly with probability of failure. This was due to the frequent use of puncture in the youngest children. The failure rate in the first series was 36%; failure rate in this series was 18%; the combined failure rate for both series was 32% (15/47). Our data indicate that we are treating patients with this abnormality at a younger age and with a greater degree of success than previously. All data comparisons are shown in Table 3.

DISCUSSION

Choanal atresia is an uncommon disorder and

TABLE 1. ABNORMALITIES ASSOCIATED WITH CHOANAL ATRESIA

Patient No.	*Associated Abnormalities*
3	Deafness; hypoparathyroidism; bladder areflexia
4	Pierre Robin syndrome; cleft palate; middle ear ossicular deformity
7	Mental retardation; chronic sinusitis and otitis, question of dyskinetic cilia syndrome
8	Mental retardation
9*	Phocomelia of left arm; ectromelia of right arm; right microphthalmia
11	Short palate; nasopharyngeal insufficiency

*Had transnasal puncture failure in previous series.

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TABLE 2. SUMMARY DATA FOR RECENT PATIENTS WITH CHOANAL ATRESIA

Patient No.	*Sex*	*Prior Surgery and Age*	*Definitive Procedure/Age (yr)*	*Pathologic Features*	*Follow-Up (yr)*	*Patency*
1	F	None	TP/0.3	B (B)	3.0	+
2	F	None	TP/ 6	L (BM)	4.6	+
3	F	Puncture at 17 days and at 3 yr	TP/ 3	B (B)	5.4	+
4	F	None	TS/ 6	L (B)	2.2	+
5	F	None	TS/ 5	L (BM)	6.3	+
6	F	None	TS/ 9	R (B)	8.8	+
7	F	Puncture, done twice	TS/ 6	L (B)	2.0	+
8	M	Puncture	TS/ 19	B (BM)	4.8	–
9	M	Puncture at 3 mo and at 2 yr	TS/ 17	B (B)	10.0	+
10	F	None	TS/ 5 LS/ 6	R (B)	9.0	+
11	M	Puncture, done three times	TS/ 6 LS/ 8	B (BM)	5.6	–

TP — transpalatal, TS — transseptal, LS — lysis scar, B — bilateral, (B) — bony, L — left, (BM) — bony and membranous, R — right, + indicates patent; – indicates stenosed.

appears to exist as a spectrum of disease, from the adult with unilateral atresia to the newborn with bilateral atresia and multiple congenital anomalies. The findings of this series support the previous association of congenital anomalies: seven of 11 patients (64%) showed physical or mental developmental abnormalities.[2] Recently a cluster of defects seen in these patients was given the acronym CHARGE: C, coloboma (ocular); H, heart defects; A, atresia choanae; R, retarded postnatal growth, development, and CNS anomalies; G, genital hypoplasia; and E, ear deformities, hearing loss.[3] Some anomalies seen in this series fit into these categories. Such associations have led to the suggestion that choanal atresia is a syndromal disorder.[4]

Although numerous theories have attempted to explain the embryologic basis for choanal atresia, none have produced an adequate explanation. Four basic theories include persistence of the buccopharyngeal membrane; persistence of the nasobuccal membrane of Hochstetter; formation of mesodermally induced adhesions at the choanae; and misdirection of mesodermal flow. Detailed discussion of the embryogenesis is beyond the scope of this paper but has been well detailed elsewhere.[5]

Since the first description of choanal atresia by Roederer more than 200 years ago, many methods of surgical correction have been described. Transpalatal, transnasal, and transseptal approaches are the most commonly used methods of repair. A transantral approach has been described but is no longer used and is only of historical note. Recent discussions have centered primarily on which approach yields best results with the least morbidity and probability of requiring revision. Each approach has proponents who have reported good results with their technique. However, many series are small and follow-up is frequently brief or incomplete.[2,6]

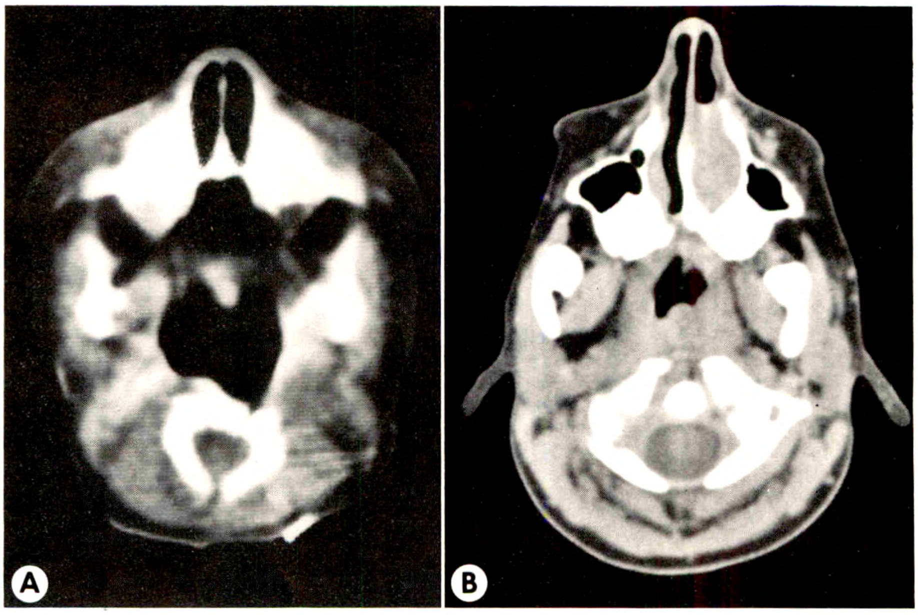
The key to successful treatment is probably not so much the surgical approach as the particular anatomic abnormality responsible for the atresia. Advances in radiography have greatly improved our diagnostic capabilities. Computed tomography defines the anatomic abnormality quite clearly and aids in selection of the best surgical approach. Determination of the type of atresia (bony, membranous, or combined) helps in deciding the amount of bony obstruction to be removed. A purely membranous atresia, which is estimated to occur in approximately 10% of cases,[7] may be treated successfully by transnasal puncture and stenting. At the other extreme, bilateral bony atresia has been characterized on CT by splaying of the posterior vomer with fusion or abutment against the posterior lateral nasal wall.[8] We call this exaggerated widening of the posterior vomer the "delta" deformity because of its triangular configuration (see Figure, A). The lateral nasal wall curves medially to varying degrees, giving an hourglass configuration to the choanae and nasopharynx (see Figure, B). An atresia with the vomerine delta deformity and lateral wall narrowing requires removal of bone to achieve a good result. Failure to remove the delta deformity adequately is probably one of the main reasons for failure of transnasal puncture.

Transnasal puncture should not be equated with all other transnasal procedures. The transnasal mi-

TABLE 3. SURGICAL METHODS FOR CHOANAL ATRESIA AND INCIDENCE OF FAILURE

Method	*Series*	*No. of Cases*	*Failures*	
			No.	*%*
Perforation	Present	5*	5	100
	Prior	18	8	44
Transpalatal	Present	3	0	0
	Prior	5	4	80
Transseptal	Present	8	2	25
	Prior	13	2	15

*Includes one transnasal puncture failure from prior series corrected transseptally in present series.



Choanal atresia. A) Bilateral bony atresia in infant with widened "delta" deformity of posterior vomer. B) Unilateral atresia with characteristic hourglass configuration of lateral nasal walls.

croscopic technique using an air drill may achieve the desired removal of the delta deformity, and it has its proponents.[9-11] We think that surgical access by this approach is too limited; however, such decisions are based on individual preference and experience.

In neonates presenting with bilateral atresia, we favor a reasonable trial of an oral airway such as the McGovern nipple. Children who do not compensate by mouth breathing require surgical intervention. Correction of unilateral atresias may be delayed until the child has developed adequately to undergo a transseptal procedure. In older children the transpalatal approach offers no better exposure than the transseptal and requires an intraoral incision. Previously, we delayed surgical repair of unilateral atresias until children were 8 years old. We have become increasingly sensitive to the social stigma for a school-age child of a chronically runny nose and have taken this into consideration in timing repair. Accordingly, successful transseptal repair in children as young as 5 years has been achieved and four of the eight children undergoing transseptal repair in this series were less than 8 years old (5, 5, 6, and 6 years). This represents a significant shift in our approach in younger children; in our previous series, no children younger than 8 years had transseptal correction.

Thin lightweight rolled Silastic sheeting was used as the stenting material of choice to the exclusion of other firm stenting materials. Experiments in dogs have shown that firm stenting materials induce fibroplasia and osteoblastic reactions at the sites of regenerating mucosa.[12] Although a few patients had restenosis despite the use of soft stents, we think that the results demonstrated in the animal model and other clinical applications merit its continued use as a scaffold for mucosal regeneration.[13]

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ISOLATED PULMONARY NODULES IN HEAD AND NECK CANCER PATIENTS

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Not infrequently, a patient with newly diagnosed head and neck cancer is noted on preoperative chest radiography to have a solitary pulmonary nodule. It is initially unclear whether the pulmonary nodule is a benign lesion or a metastatic or primary lung malignancy. Considerable controversy exists regarding the evaluation of such patients as well as the treatment, assuming that the pulmonary lesion is malignant. We have reviewed the UCLA experience with patients who had head and neck cancers and pulmonary cancers no more than 5 years apart, and reviewed the literature on early stage lung cancer. We present a rational approach to the workup and treatment of patients with head and neck cancer and a pulmonary nodule on chest radiography.

KEY WORDS — head and neck cancer, pulmonary nodules.

INTRODUCTION

During the evaluation of a patient with newly diagnosed head and neck cancer, the chest radiograph occasionally reveals isolated or multiple pulmonary nodules. When multiple nodules are present, the patient most likely has metastatic disease. In such patients, major head and neck surgery generally is contraindicated. Further study will be necessary to evaluate the role of thoracic surgery in the resection of multiple lung metastases from head and neck primary tumors.

The optimal course of treatment is less clear when the chest radiograph in a patient with newly diagnosed head and neck cancer reveals an isolated pulmonary nodule. Multiple primary carcinomas of the aerodigestive tract are well documented,[1-10] and therefore the possibility of malignancy must be strongly entertained. Histologic diagnosis of the lung lesion is usually available, but often not without significant delay and occasional morbidity; some lesions can be diagnosed only at thoracotomy. It is unclear whether head and neck procedures should be delayed to obtain a tissue diagnosis of the lung lesion.

Most surgeons advocate the aggressive sequential surgical treatment of both tumors in patients presenting synchronously with head and neck and lung cancers.[11] Marks and Schecter,[5] as well as other authors,[2,11] state that the bronchogenic carcinoma is more lethal and should be treated first except in the presence of airway obstruction. However, when a lung cancer presents as a solitary pulmonary nodule, the head and neck cancer is generally more lethal, as we shall discuss. In addition, a certain percentage of such patients will have pulmonary lesions that prove to be benign. In such patients, much time will have been expended unnecessarily prior to treatment of the head and neck cancer.

Finally, there is little information in either the head and neck or thoracic surgery literature documenting the outcome of sequential surgical resection of head and neck and lung carcinomas.

The purpose of this paper, therefore, is to determine the best course of treatment for the patient with newly diagnosed head and neck cancer and an undiagnosed isolated pulmonary nodule. Is a histologic diagnosis of the pulmonary lesion necessary prior to treatment of the head and neck cancer? Is sequential surgical resection of the two lesions indicated, and, if so, in which order? In which patients is resection contraindicated?

We have attempted to answer these questions by reviewing the UCLA experience since 1960 in patients who had major head and neck operations and thoracotomy for lung cancers within a 5-year period. We have also reviewed the literature regarding pulmonary nodules in terms of differential diagnosis, evaluation, and survival rates following resection of malignant lesions. On the basis of the experience at UCLA and the literature, we present a rational approach to the surgical treatment of the head and neck cancer patient with an undiagnosed pulmonary nodule.

MATERIALS AND METHODS

To address the issue of survival among patients with head and neck and lung carcinomas, we recovered charts from the UCLA Department of Pathology and the UCLA Tumor Registry that described patients with both cancers. There were 163 charts

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TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS REVIEWED

		Squamous Cell Carcinoma of Head and Neck		*Lung Tumor*		
Patient No.	*Birth Date*	*Date of Diagnosis and Location*	*Treatment*	*Date of Pathologic Examination and Diagnosis*	*Treatment*	*Follow-Up*
1	4/15/15	9/77 True vocal cord	L hemilaryngectomy	10/78 RLL squamous cell carcinoma	Lobectomy	10/78 Died of post-operative bleeding
2	8/17/20	12/81 True vocal cord	Total laryngectomy	3/80 LUL squamous cell carcinoma	Lobectomy	11/85 Alive and well
3	6/18/24	9/80 Palate	Maxillectomy and palatectomy	11/80 RUL bronchoalveolar carcinoma	Lobectomy	7/82 Died with active head/neck disease
		8/81 Palate recur	Immunotherapy			
4	3/30/30	10/83 Soft palate	7,000 cGy	8/84 LUL adenocarcinoma	Lobectomy	1/86 Alive without recur
		5/84 Palate recur	Composite and LRND			
5	11/27/24	10/83 True vocal cord	Total laryngectomy	8/82 R middle lobe squamous cell carcinoma	Lobectomy	9/84 Died with lung disease
				6/83 Lung recur	R radical pneumonectomy	
6	11/16/09	11/76 Epiglottis	6,000 cGy	7/76 RLL adenocarcinoma	Lobectomy	7/79 Died with lung disease
		7/77 Local recur	Total laryngectomy			
7	8/13/97	8/63 Epiglottis	Total laryngectomy	9/62 RLL undifferentiated carcinoma	Lobectomy	6/64 Died with lung disease
8	10/ 5/11	5/80 True vocal cords	Laryngectomy and LRND	3/80 LUL squamous cell carcinoma	Lobectomy	8/81 Died with lung metastasis
		2/81 Neck recur		10/80 RLL squamous cell carcinoma	Wedge resection	
9	8/25/01	10/71 True and false vocal cords	Total laryngectomy	8/72 LUL squamous cell carcinoma	Lobectomy	11/73 Died with lung metastasis
		8/72 Neck recur	LRND	1/73 Bilateral metastasis		
10	3/ 4/23	4/80 L floor mouth and true vocal cord	Composite and total laryngectomy and LRND	9/81 RUL squamous cell carcinoma	Wedge resection	4/83 Died with head/neck recur
		5/82 Local recur	Resection			
11	4/25/05	11/72 Tongue	Partial glossectomy and LRND	6/74 LUL squamous cell carcinoma	Lobectomy	8/74 Died with pancreatic cancer
12	10/15/28	2/83 Vallecula	Supraglottic laryngectomy	1/83 Squamous cell carcinoma	Wedge resection	9/85 Alive and well
13	11/23/00	6/81 Larynx	Total laryngectomy	7/81 RUL squamous cell carcinoma	Lobectomy	2/84 Died of lung metastases
		12/82 Neck recur	RRND	2/84 Multiple metastases		
14	4/20/30	3/81 Larynx	Supraglottic laryngectomy and RRND	5/82 RUL and RLL squamous cell carcinoma	Bilateral lobectomy	5/85 Alive and well
15	9/21/20	1/76 Soft palate	5,400 cGy	11/81 RUL squamous cell carcinoma	Wedge	4/82 Died with head/neck disease
		4/80 Neck recur	RRND			
		10/81 Oral recur				
16	2/ 9/08	5/82 Retromolar trigone	Composite and LRND	5/82 LUL squamous cell carcinoma	Lobectomy	4/85 Alive and well
17	7/ 7/02	5/65 Soft palate and tongue base	Composite and LRND	3/68 RUL adenocarcinoma	Lobectomy	1/73 Died of esophageal cancer

recur — recurrence, cGy — centigray, LRND — left radical neck dissection, RRND — right radical neck dissection, RLL — right lower lobe, LUL — left upper lobe, RUL — right upper lobe.

recovered, and these were examined for patients meeting the following criteria:

1. Both malignancies must have occurred within a 5-year period.
2. The lung lesions must have presented as a solitary pulmonary nodule noticed incidentally on radiography.
3. Both cancers must have been treated surgically with attempt at cure.
4. Only squamous cell carcinomas of the head and neck were considered.

In this manner 17 patients were identified and their charts were examined in order to determine survival among this select group of patients.

TABLE 2. HISTOLOGIC DIAGNOSIS OF 955 RESECTED PULMONARY NODULES

Malignant lesions (49.1%)	
Bronchogenic carcinoma	38.1%
Squamous cell	17.9%
Adenocarcinoma	13.3%
Bronchoalveolar	2.3%
Others	4.6%
Metastatic cancer	9.3%
Others (sarcoma, plasmacytoma, Hodgkin's disease, teratoma)	1.8%
Benign lesions (50.9%)	
Benign tumors (chondroma, mesothelioma, adenoma, neurogenic tumor)	13.8%
Tuberculosis	26.6%
Others (abscess, aspergilloma, bronchogenic cyst, echinococcus cyst, diaphragmatic hernia, others)	13.5%

Data from Toomes et al.[18]

CASE REPORTS

Case 4. A 53-year-old woman presented to the UCLA Medical Center in October 1983 with a T1N0M0 squamous cell carcinoma of the left retromolar trigone. She initially was treated with 7,020 cGy to the primary tumor site. In May 1984 she was found to have a local recurrence and was scheduled for a composite resection and left radical neck dissection. On preoperative chest radiography, however, she was found to have a left upper lobe lung nodule. A thoracic surgery consultant recommended resection of the head and neck lesion prior to further chest evaluation. She therefore underwent resection on May 17, 1984, and recovered uneventfully.

One month following discharge, the patient underwent thoracotomy. A left lingular resection was performed for adenocarcinoma. Because of positive surgical margins on pathologic examination, she required a completion left upper lobectomy soon thereafter. As of January 1986 she was in good health without evidence of recurrence.

Case 12. A 54-year-old woman presented to an outside hospital in January 1983 with hoarseness and dysphagia and was found to have a clinically T2N2M0 squamous cell carcinoma of the left vallecula. Preoperative screening chest radiography revealed a small coin lesion of the right upper lobe. A computed tomography–guided fine-needle aspiration of the lung lesion was performed and revealed carcinoma. After the aspiration, she developed a pneumothorax requiring chest tube insertion. The patient then was transferred to the UCLA Medical Center.

A CT scan of the chest revealed no evidence of hilar or mediastinal disease. Thoracic surgery consultation was obtained; the consultant advised resection of the vallecular lesion first, as he believed that this was the more lethal lesion. The patient therefore underwent a supraglottic laryngectomy and left radical neck dissection in February 1983.

After an uneventful recovery, she underwent a right upper lobe wedge resection in March 1983. No disease was found in the hilum or mediastinum; final pathologic diagnosis revealed bronchoalveolar carcinoma. The patient subsequently received 5,900 rad to the head and neck tumor site. When last seen in September 1985, the patient was doing well without evidence of head and neck or lung recurrence.

RESULTS

Table 1 lists the clinical characteristics of the 17 patients who fulfilled the criteria for our analysis. The patients had the following head and neck tumors: ten had laryngeal cancers, six had oral cavity lesions (four of the palate, one of the tongue, and one of the retromolar trigone), and one had simultaneous lesions of the floor of mouth and larynx. Of the lung tumors, 12 were epidermoid carcinoma, three were adenocarcinoma, one was undifferentiated, and one was bronchoalveolar. Eight of the lesions presented synchronously, and nine metachronously.

One patient (No. 14) was found to have disease in both the right upper and lower lobes at the time of thoracotomy, and bilateral lobectomy was performed.

Of the patients in Table 1, five are currently alive without evidence of recurrence. One died 5 years after his last operation of an unrelated cause and therefore is considered cured. Two died of unrelated causes within a year of their second resection. Therefore, the overall cure is six of 15 patients or 40%. Three patients died with head and neck cancer, and six with lung cancer. The 1-year survival of all 17 patients, calculated from the time of the second operation in the case of metachronous lesions, is 13/17 (76.5%). The 3-year survival is 5/16 (31.3%); one patient (No. 4) is alive and well with less than 3 years' follow-up. The review was completed in August 1986.

DISCUSSION

The analysis of this series of patients was designed to assist the head and neck surgeon in the management of patients with upper aerodigestive tract cancers who are noted incidentally to have solitary pulmonary nodules on chest radiography. Because of the difficulty in retrospectively identifying that group of patients, it was decided to review those head and neck cancer patients who also had solitary pulmonary nodules resected that proved to be malignant. It is, after all, this group of patients in whom we are most interested, since there is no debate regarding treatment if the pathologic diagnosis reveals the lung lesions to be benign.

By convention, multiple primary tumors are considered synchronous if detected within 6 months of each other.[3] It was necessary for this study to consider patients whose lesions were within 5 years of each other in order to identify an adequate number of patients. The conclusions derived from such patients are useful in designing a strategy for the management of the specific patients in whom we are most interested, namely, those head and neck cancer patients with a synchronous undiagnosed pulmonary nodule. Specifically, we feel that the survival rate of 40% suggests that sequential resection of the head and neck and lung cancers is beneficial.

At first glance, one might be surprised to find such high survival rates in patients with relatively lethal cancers. It is important to emphasize, therefore, that this is a select group of lung cancer patients in that they all presented with the incidental chest radiograph finding of an isolated pulmonary nodule. Apparently, presenting with a head and neck cancer prompted chest radiographic examinations that otherwise would not have been performed, contributing to the early detection of the lung lesions.

Our results are not inconsistent with reported survival rates of patients with isolated lung cancers. Martini and Beattie,[12] in 1977, reported a series of 198 patients with postoperative stage I lesions; the 3-year survival rate was 77%. This survival rate represented an improvement over the findings of Jackman et al,[13] who in 1969 found a 5-year survival rate of 51.3% in a similar group of patients.

Therefore, given a patient with resectable cancers in the head and neck and in the lung, it is worthwhile to pursue sequential surgical resection. The next issue to be addressed is which lesion should be resected first. Other authors have stated that the bronchogenic carcinoma should be operated upon first.[2,5,11] It is our belief, however, that the head and neck cancer should be resected first in those patients with head and neck cancers and isolated pulmonary nodules. The reasons have been outlined by one of us (T.C.C.)[11] and include the following: 1) as opposed to lung cancers in general, the isolated pulmonary nodule is usually less lethal than the head and neck cancer; 2) the patient will have better pulmonary function intraoperatively and postoperatively for the head and neck procedure if that operation is done before lung resection; 3) in most cases, the recovery time following a head and neck operation is significantly shorter than that following thoracotomy and therefore the interval between the operations would be shorter; and 4) the head and neck operation should not be delayed by the workup of the pulmonary lesion, which may prove to be benign.

Most surgeons feel that the head and neck cancer is unresectable when metastatic to the lung. Although it is not always possible to determine whether a solitary nodule is primary or metastatic, several criteria are helpful. When the histopathologic results differ, the lesions are clearly separate primary tumors. It is rare to find metastatic lung disease in the absence of cervical adenopathy. Multiple lung lesions suggest metastatic disease, although small cell carcinoma can present in this fashion, and a tissue diagnosis should be made so that appropriate chemotherapy may be begun.[4] Finally, on pathologic analysis, adjacent areas of endobronchial carcinoma in situ suggest a lung primary tumor.

Whether the lung lesion is metastatic or not is inconsequential to the clinician. The solitary lung lesions should be resected, because a primary lung carcinoma cannot be distinguished absolutely from a metastatic lesion.

Next we will discuss the recommended workup of patients who present with coin lesions. The critical issue in lung cancers is resectability, since there are only rare cures without resection.[14] It is therefore essential to attempt to eliminate contraindications to resection of the lung lesion. These include contralateral mediastinal disease, bilateral lesions, malignant pleural effusion, nerve involvement such as phrenic or vagal paralysis, tumor within 2 cm of the carina, and distant metastasis.

The chest CT scan is a reliable method of ruling out unresectable disease prior to thoracotomy, particularly in the mediastinum. Breyer et al[15] found that chest CT had a sensitivity of 82% and a specificity of 100% for mediastinal disease when conclusive. They suggest that for a patient with negative results on CT, the surgeon may omit mediastinoscopy in staging. Thoracic CT scans are also effective in demonstrating multiple nodules or bilateral disease in a patient thought to have a solitary lesion.[14]

The above contraindications to resection can be evaluated by physical examination with careful attention to neurologic examination, CT scanning of the chest, chest radiography, and liver function tests. Pulmonary function tests and an arterial blood gas analysis should be performed to assess the patient's medical candidacy for thoracotomy.[16] A tuberculin skin test may help guide the further workup of the lung nodule and should be performed.

Although some clinicians perform liver/spleen, brain, or bone scans, Ramsdell et al[17] found that of 131 patients with bronchogenic carcinoma with no evidence of metastatic disease on routine studies and physical examination, only one case of metastatic disease was found.

The differential diagnosis of an isolated pulmonary nodule includes, in addition to malignancy, many other possibilities. In a study of 955 resected coin lesions of the lung, Toomes et al[18] found malignancies in 49%. Their results are summarized in Table 2 to suggest the variety of lesions that may present as solitary nodules. As most head and neck cancer patients have a significant smoking history, the likelihood of malignancy in these patients is greater. Malefatto et al[4] found that in 36 head and neck cancer patients with solitary nodules on chest radiography, 53% of the nodules represented second primary lung carcinomas, 19% were metastatic, and 28% were benign.

What are the available alternatives in pursuing pathologic diagnosis of the lung lesion? Sputum cytology is not particularly useful in diagnosing bronchogenic carcinoma in this group of patients, because of the possibility of contamination from the head and neck tumor. Bronchoscopy with brushings and transbronchial biopsies may obtain a diagnosis in up to 79% of patients.[19] Transthoracic fine-needle aspiration under CT[20] or fluoroscopic[21] guidance is frequently diagnostic in peripheral lesions but is associated with a 27% incidence of pneumothorax (with 14% of the cases of pneumothorax requiring chest tube drainage).[14] In neither the transthoracic nor the transbronchial method can a negative result rule out malignancy. Sagel et al[21] state that the true negative rate in transthoracic aspiration is only 87%; ie, given a negative result on aspiration, there is still a 13% chance of malignancy.

Besides adding time and potential morbidity to the workup, the above tests may be inconclusive, in which case the patient still must undergo thoracotomy. Therefore, given an isolated, resectable pulmonary nodule in a patient with head and neck cancer, and assuming as stated above that the head and neck cancer should be treated first, it is not worthwhile delaying treatment of the head and neck cancer in order to pursue histologic diagnosis of the pulmonary lesion. In fact, contrary to intuition, it is only important to obtain tissue diagnosis of the pulmonary lesion when radiographic studies indicate that the lesion is not resectable. In this instance one would want to establish that the patient had an unresectable lung lesion prior to treating the head and neck cancer.

The possibility exists that the lung cancer could be small (oat) cell carcinoma. Although the role of surgery in small cell cancer has not been investigated fully, there have been long-term survivors with a combined therapy (surgery and chemotherapy), and this approach currently is recommended for isolated lesions.[14,22] Thus the possibility of finding small cell carcinoma does not influence our strategy in these patients.

To summarize, the following approach to the patient with newly diagnosed head and neck cancer and an isolated pulmonary nodule is recommended, on the basis of the patients described herein as well as a review of the literature. A complete history and physical examination are necessary, with special emphasis on the identification of contraindications to resection, such as superior vena cava syndrome, vocal cord paralysis, and others. Liver function, blood gas, and pulmonary function tests and skin tests for tuberculosis should be performed. Lastly, on the basis of the evidence presented above, we believe that a thoracic CT scan is a useful, noninvasive method of evaluating the mediastinum and lung fields and should be a part of the workup in the head and neck cancer patient with a lung lesion.

Assuming that there is no evidence of metastatic disease or unresectable disease, the patient should be informed that there is a possibility of malignant pulmonary disease and that the only reasonable chance for cure is surgical resection of both lesions. If the patient understands and wishes to proceed, the head and neck cancer should be resected prior to further evaluation and treatment of the pulmonary nodule.

In conclusion, head and neck surgeons occasionally are presented with an isolated pulmonary nodule on the chest radiograph of a patient with a newly diagnosed head and neck cancer. There is a dearth of literature addressing the best manner in which to proceed in these cases. We have attempted to delineate a reasonable approach to such patients based upon the UCLA experience and a review of the literature. In closing, we agree with Cahan and Montemayor,[2] who stated in 1962, "This finding of a solitary lung shadow with a cancer elsewhere, although of real concern, does not necessarily represent a hopeless situation but may in fact have its own virtue." The major virtue is the early detection of an otherwise too often lethal disease.

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EFFECTS OF CRICOTHYROID MUSCLE CONTRACTION ON LARYNGEAL RESISTANCE AND GLOTTIC AREA

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To determine the functional significance of the cricothyroid muscle (CT) in respiration, laryngeal resistance was measured in anesthetized dogs, along with electromyographic activity of the posterior cricoarytenoid muscle (PCA) and CT. In two dogs the larynx was videotaped simultaneously via a telescope. Increased CT activity was induced by airway occlusion or hypercapnia. Observations were carried out before and during cold blockade of the recurrent laryngeal nerve (RLN) or the nerve to the CT (external branch of the superior laryngeal nerve [Ext SLN]). Paralysis of the CT had no effect on laryngeal resistance or glottic area, even at very high levels of CT activity. Blockade of the RLN increased inspiratory resistance, but did not have a significant effect on expiratory resistance. Electrical stimulation of the Ext SLN produced tetanic CT contraction, which increased laryngeal resistance in both inspiration and expiration. This was true even at very high levels of PCA activity. These results indicate that although artificially induced CT contraction markedly affects laryngeal resistance, physiologic levels of respiratory activity do not have a significant effect.

KEY WORDS — laryngeal muscles.

INTRODUCTION

Respiratory activity of the cricothyroid muscle (CT) has been documented clearly by several investigators.[1-9] The functional significance of this activity is, however, not well understood.

In general the CT contracts phasically in inspiration during anesthesia and slow wave sleep.[1-9] Respiratory stimuli, such as airway occlusion or hypercapnia, increase the inspiratory activity and can elicit contraction during expiration as well.[4,6-11] The behavior of this muscle varies with level of consciousness.[4,10] During alert wakefulness in rats, CT activity is very minimal and primarily expiratory.[4,12] In conscious humans, CT activity is generally absent during quiet breathing, but respiratory activity can be recruited by hypercapnia or by voluntary deep inspiration (Woodson, unpublished data). Thus, although the CT behaves primarily as an inspiratory muscle during anesthesia and sleep, it may have a different function during wakefulness.

The effect of electrically induced CT and posterior cricoarytenoid muscle (PCA) contraction on laryngeal resistance was studied by Konrad and Rattenborg[13] in the isolated in situ larynx of dogs, as well as in the excised larynx. They found that CT contraction alone produced laryngeal adduction and an increase in resistance. Simultaneous CT and PCA stimulation led to a lower laryngeal resistance than that observed when only the PCA was stimulated. They concluded that the CT can either decrease or increase laryngeal resistance, depending on the presence or absence of concurrent PCA activity.

Previous research by Dedo[14] suggests that CT contraction has a significant effect on vocal fold position. In dogs, following unilateral recurrent laryngeal nerve (RLN) transection, the vocal fold assumed an adducted position. The adduction was attributed to the CT, since this is the only laryngeal muscle not supplied by the RLN, and because the vocal fold moved laterally following denervation of the CT.

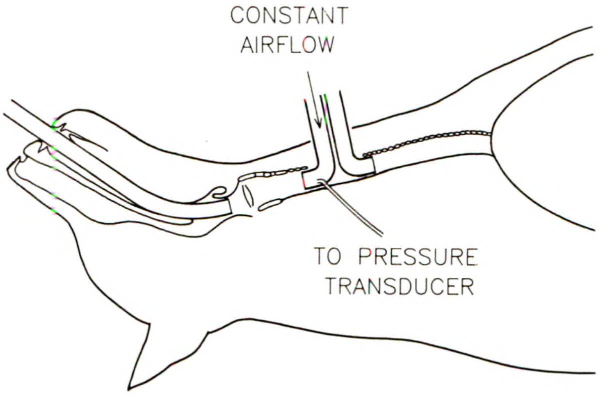

Fig 1. Dog breathes spontaneously through lower tracheal cannula. Constant stream of warm, humidified air is passed into upper tracheal cannula and flows through larynx in expiratory direction. Pressure transducer connected to upper tracheal cannula measures subglottic pressure.

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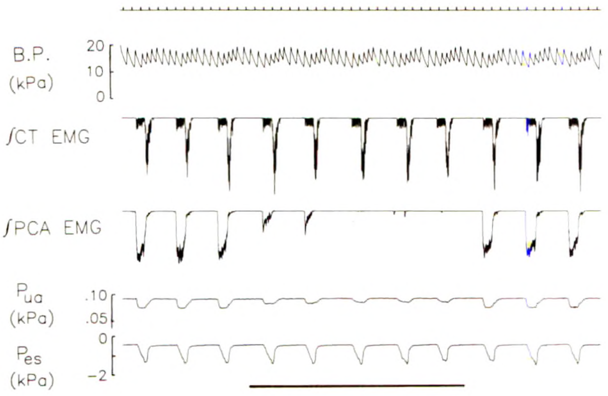

Fig 2. Effect of recurrent laryngeal nerve (RLN) blockade (heavy line) on laryngeal resistance. Traces are, from top to bottom: time marker in seconds, blood pressure (B.P.), integrated cricothyroid muscle electromyogram (∫ CT EMG), integrated posterior cricoarytenoid muscle electromyogram (∫ PCA EMG), pressure in upper airway (Pua), and esophageal pressure (Pes). kPa — kilopascal. With RLN blockade laryngeal resistance during inspiration decreased less than under control conditions. Note that CT contraction in absence of PCA activity does not result in any increase in laryngeal resistance.

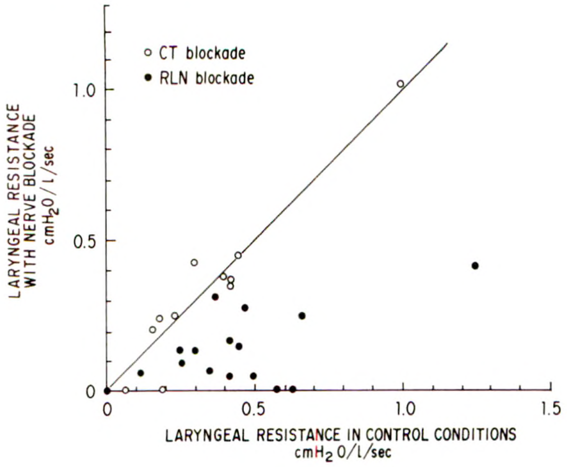

Fig 3. Inspiratory decrease in laryngeal resistance during blockade of recurrent laryngeal nerve (RLN) and external branch of superior laryngeal nerve, plotted against corresponding value before blockade, for all breathing conditions. Note that open circles cluster along 45° line, indicating little or no effect of removal of CT activity. Closed circles, however, fall below line, suggesting that muscles innervated by RLN (eg, posterior cricoarytenoid muscle [PCA]) are responsible for decreased laryngeal resistance during inspiration.

The CT is also active in phonation.[15] It is usually quiet during low frequency phonation, but contracts strongly in falsetto or upper register phonation. This suggests that its primary role in phonation is to increase tension in the vocal fold. In his study of CT function, Arnold[2] concluded that the primary function of the CT is phonatory, and that its respiratory activity is not significant.

The present study was designed to determine whether or not physiologic levels of CT contraction with respiration have a significant influence on laryngeal resistance.

METHODS

Ten healthy mongrel dogs (10 to 16 kg in weight) were anesthetized with an intravenous injection of alpha-chloralose (0.1 g/kg) and urethane (1.0 g/kg). Catheters were placed in the femoral artery and vein to monitor blood pressure and permit additional administration of anesthetic, respectively. An anterior longitudinal midline cervical incision was made to expose the larynx and trachea. The RLNs and the external branches of both superior laryngeal nerves (Ext SLNs) were identified and isolated. A large tube was placed through the mouth with the tip just below the epiglottis to eliminate fluctuations in resistance above the larynx (Fig 1). A saline-filled polyethylene catheter was placed in the esophagus and connected to a Statham P50 pressure transducer to monitor intrathoracic pressure changes associated with breathing. The trachea was incised and the caudal segment intubated to permit spontaneous breathing. Another cannula was placed in its rostral segment and used to pass a constant flow of warm (37°C) humidified air (0.34 to 0.40 L/s) through the larynx in an expiratory direction (Fig 1). The cannulas were secured with circumtracheal ligatures, with care taken not to injure the laryngeal nerves. Subglottic pressure was measured with a differential pressure transducer (Statham PM 5 ETC) and used to calculate resistance.

Hooked wire electrodes were placed in the CT and PCA, and the resultant signals were filtered, amplified, rectified, integrated, and displayed on a strip chart recorder (Gould Brush 2600), along with esophageal and subglottic pressures. These parameters were recorded during quiet spontaneous breathing, brief tracheal occlusions, and hypercapnia (10% carbon dioxide in oxygen) in the neurally intact dog and during cold blockade of either the RLN or the Ext SLN.[16]

In two of the dogs, glottic area also was evaluated by measuring the image obtained by simultaneous telescopic videolaryngoscopy.

In four dogs, the Ext SLNs were stimulated electrically with square pulses sufficient to elicit strong contraction of the CT (0.22 to 1.00 V, 0.1-ms duration, 40 Hz; 40 Hz has been found to be the fusion frequency for the canine CT[17]).

Laryngeal resistance was calculated as the ratio between subglottic pressure and the constant airflow through the larynx. Results from multiple trials were averaged for each dog. Data obtained in control conditions and during blockade of either the RLN or the Ext SLN were compared by use of a paired t test. Changes were considered significant at $p < .05$.

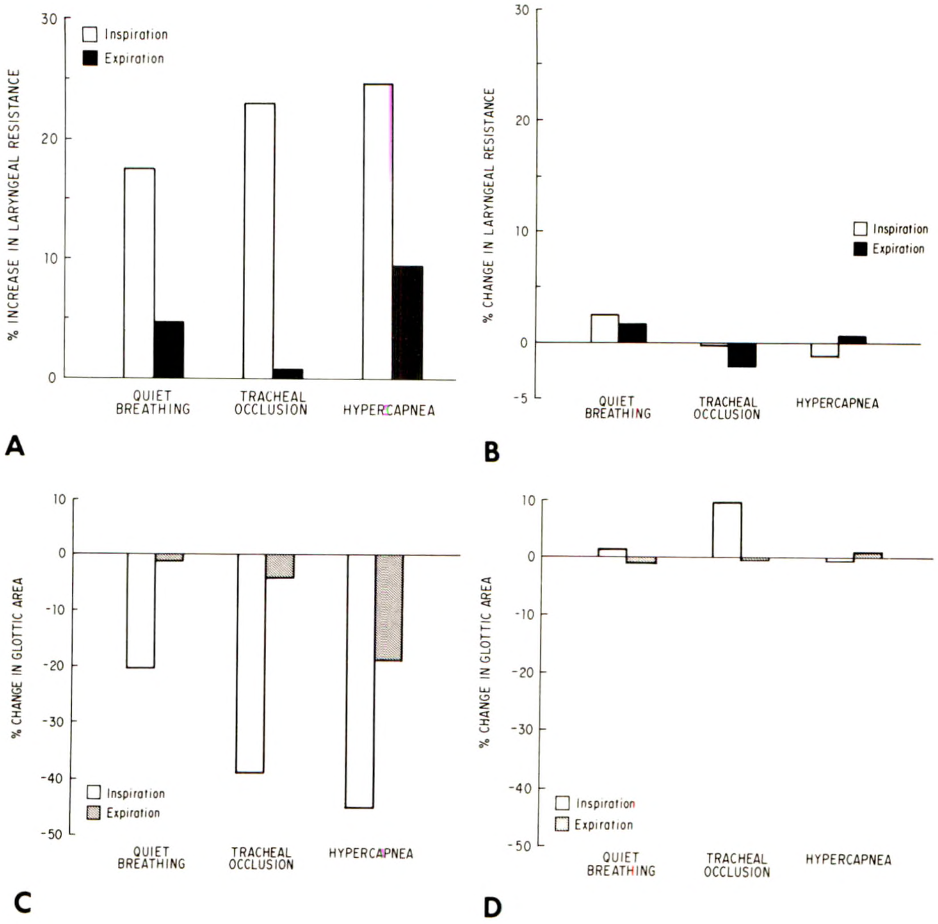

Fig 4. Effect of blockade of A) recurrent laryngeal nerve (RLN) and B) external branch of superior laryngeal nerve (Ext SLN) on average laryngeal resistance and C) RLN and D) Ext SLN on average glottic area (as observed by videoendoscopy). Values are expressed in terms of percent difference from values in intact animal.

RESULTS

In quiet breathing laryngeal resistance decreased during inspiration; these resistance changes were magnified by tracheal occlusion and hypercapnia. Blockade of the RLN greatly diminished the phasic changes in resistance in all three experimental conditions. Figure 2 illustrates the effect of RLN blockade on laryngeal resistance during quiet breathing in one trial. The overall results are summarized in Figs 3 and 4A. In Fig 3 the inspiratory decreases in laryngeal resistance in the neurally intact dogs (all experimental conditions) are plotted against the corresponding values during RLN blockade. Figure 4A represents the average changes in laryngeal resistance with RLN blockade, in both inspiration and expiration, under each experimental condition. Only the changes in inspiratory resistance were statistically significant.

Videolaryngoscopy revealed that RLN blockade reduced the phasic changes in glottic area. The average changes in glottic area during RLN blockade are shown in Fig 4C.

Blockade of the CT did not alter laryngeal resistance, as illustrated in Fig 5A. Overall data pertaining to CT blockade on laryngeal resistance and glottic area are shown in Figs 3 and 4B,D.

Electrical stimulation of the Ext SLN resulted in a tetanic contraction of the CT that increased laryngeal resistance during both inspiration and expiration. The difference between inspiratory and expiratory resistances was larger than in the control condition (Fig 5B, left versus right panel).

DISCUSSION

These results indicate that in anesthetized dogs, spontaneous inspiratory CT contraction does not significantly affect laryngeal resistance. In fact, even when the muscle was contracting very strongly in response to respiratory stimuli, temporary paralysis of the CT produced no change in laryngeal resistance (Fig 4A,B). During cold blockade of the RLNs, when the CT was the only intrinsic laryngeal muscle still active, the phasic decrease in inspiratory laryngeal resistance was reduced markedly. The residual fluctuations in resistance could not be attributed to the CT, since Ext SLN blockade did not alter laryngeal resistance (Fig 5A). It is more likely that the residual changes in resistance were due to traction exerted on the larynx by the respiratory movement of the trachea. In fact, stabilization of the trachea minimized these fluctuations. Moreover, downward traction on the trachea

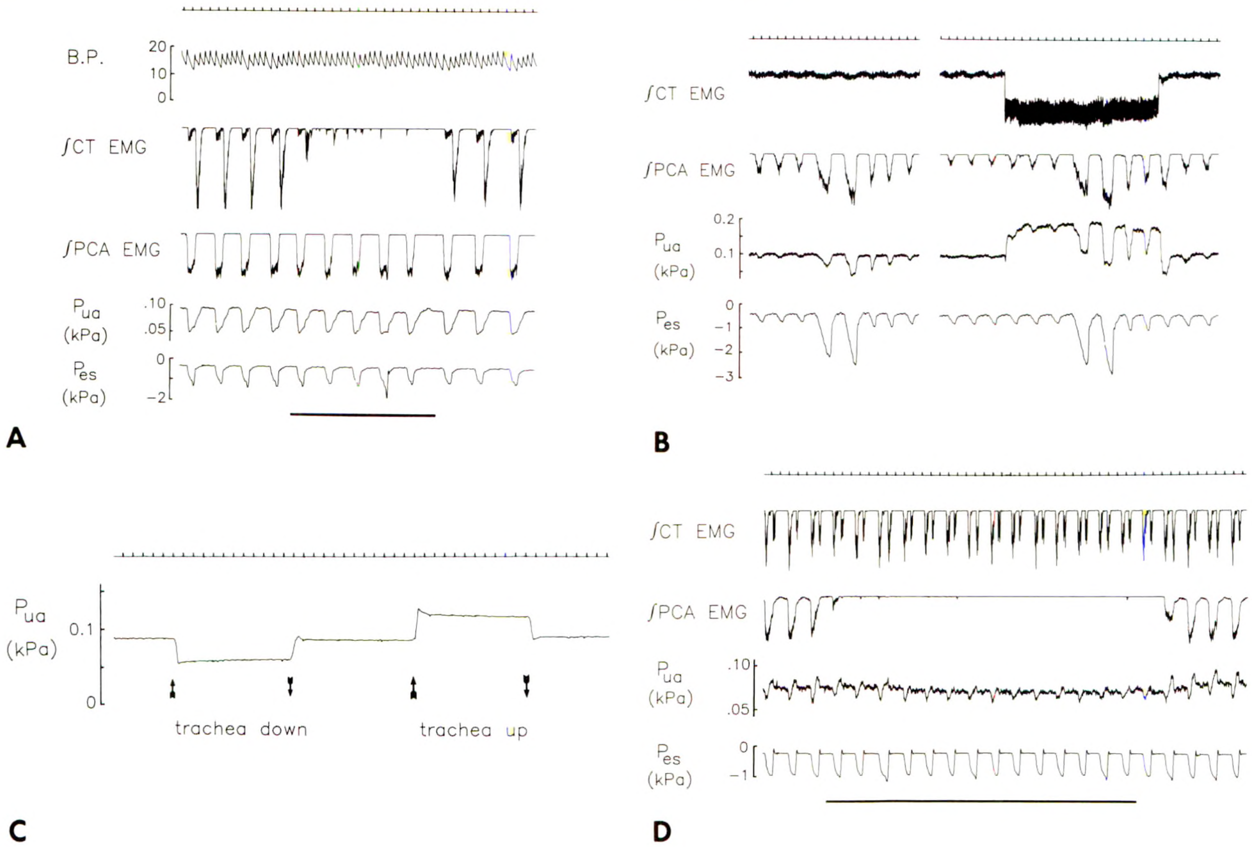

Fig 5. Traces as in Fig 2. A) Effect of blockade (heavy line) of external branches of superior laryngeal nerves (Ext SLNs) (cricothyroid muscle [CT] paralysis). Note that CT blockade does not alter phasic changes in laryngeal resistance. B) Effect of electrical stimulation of Ext SLNs (CT contraction) on laryngeal resistance. Tracheal occlusion, indicated by larger swings in esophageal pressure, is performed for two respiratory cycles before producing increased inspiratory contraction of posterior cricoarytenoid muscle (PCA; left panel) and during (right panel) electrical stimulation of CT. Electrical stimulation produces increased laryngeal resistance, both in inspiration and expiration. Note that, at any level of PCA activity, laryngeal resistance does not decrease during inspiration to level seen in control condition (left panel). C) Effect of tracheal displacement on laryngeal resistance. Maneuvers are performed in absence of any muscle activity (post mortem). Note that downward displacement decreases laryngeal resistance, whereas upward displacement increases it. D) Effect of recurrent laryngeal nerve (RLN) blockade, (heavy line) in presence of phasic expiratory augmentation of laryngeal resistance. Note that RLN blockade reduces both inspiratory decreases and expiratory increases in laryngeal resistance. Effect on expiratory resistance is particularly evident. Note also that during blockade there is downward shift of subglottic pressure suggesting removal of tonic adductor activity.

in the absence of any muscle activity significantly reduced laryngeal resistance (Fig 5C).

Blockade of the RLN and Ext SLN in the three experimental conditions produced no statistically significant effect on expiratory laryngeal resistance (Fig 4A,B), although a slight increase in expiratory resistance following RLN blockade was present, especially during CO_2 stimulation. This effect, however, could be due to withdrawal of postinspiratory PCA activity, rather than active CT contraction in expiration.

The Konrad and Rattenborg hypothesis predicts that CT contraction alone would result in an increase in laryngeal resistance.[5,13] On the contrary, our results indicate that spontaneous CT contraction had no significant effect on laryngeal resistance at any level of respiratory stimulation.

Electrically induced strong CT contraction did indeed produce laryngeal adduction and increased laryngeal resistance, as previously reported by Konrad and Rattenborg.[13] Hence, the present results do not invalidate this mechanical effect of strong CT activity, but indicate that the spontaneous level of CT activity is insufficient to affect resistance. We did not observe a synergistic effect of CT on PCA function in decreasing laryngeal resistance, as reported by Konrad and Rattenborg. In fact, although greater fluctuations in laryngeal resistance were present during electrical stimulation of CT, the absolute value of inspiratory laryngeal resistance was always higher than in control conditions. This was true even when PCA activity was augmented markedly by tracheal occlusion. Differences in experimental conditions could account for this discrepancy. For instance, in our experiment the PCA was not stimulated electrically.

The greater inspiratory decrease in laryngeal resistance during CT electrical stimulation could be due to the tension-length relationship of the PCA.

If, when stretched by CT contraction, PCA sarcomeres were closer to optimal sarcomere length, subsequent PCA activation would result in greater tension. An alternative explanation is that a given displacement of the vocal fold produces a much greater relative change in resistance when the folds are in a more adducted position.

In one dog, strong expiratory CT activity was observed during light anesthesia, and this was associated with an increase in laryngeal resistance during expiration. However, the fact that RLN blockade greatly reduced the expiratory resistance (Fig 5D) indicated that laryngeal adductors, such as the thyroarytenoid and lateral cricoarytenoid muscles, were primarily responsible. The residual increase in expiratory resistance possibly could be due to CT contraction; alternatively, the cranial displacement of the larynx in expiration also could be a factor, as shown in Fig 5C.

The present results appear to conflict with the findings of Dedo,[14] who reported that vocal fold position immediately following RLN transection in dogs was dependent on the neural status of the CT. The position was paramedian with the CT intact, and more lateral following denervation of the CT. In his study, however, vocal fold position was observed during phonation under very light anesthesia. Moreover, the experimental observations were based on subjective observation and photography of subtle differences, rather than objective quantification of laryngeal resistance or glottic area. There was no electromyographic documentation of active CT contraction during this phase of the experiment. His findings are best compared to the observation in the present study of increased laryngeal resistance during expiratory contraction, possibly a phonatory activity. Our results would suggest that immediately following RLN paralysis, vocal fold position would be intermediate during inspiration, but possibly paramedian in phonation.

Numerous reports, which document that vocal fold position in laryngeal paralysis can be correlated with site of lesion, support the hypothesis that spontaneously occurring CT contraction is capable of adducting the larynx.[14,18-20] In patients with bilateral RLN paralysis and airway obstruction, paralysis of the CT muscle has been suggested as a means to achieve a wider glottis.[21,22] Although this study indicates that under normal conditions, and in acute RLN paralysis, spontaneous respiratory CT activity is insufficient to adduct the vocal fold, it is possible that chronic RLN paralysis could induce some change in CT activity. Vocal fold position frequently has been reported as intermediate immediately following RLN paralysis, with gradual abduction over months.[19,20] This would be consistent with an increased strength or mechanical effect of CT contraction over time after RLN paralysis.

Activity of the CT is affected markedly by level of consciousness, and comparisons of CT activity in anesthetized dogs to that in conscious or sleeping humans must be made with caution. Studies of CT function in rats, however, indicate that CT inspiratory activity is similar during anesthesia and slow wave sleep, and minimal during wakefulness.[4,12] It seems therefore unlikely that inspiratory CT contraction in any physiologic condition would be stronger than that observed in this study.

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THE SIXTH WORLD CONGRESS FOR BRONCHOLOGY

The Sixth World Congress for Bronchology will be held October 18-20, 1989, in Tokyo, and October 20-22, 1989, in Kyoto, Japan. For further information, contact Dr Masayuki Niitsuma, Secretary General, 6th World Congress for Bronchology, Department of Surgery, Tokyo Medical College, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Japan; telephone 03-342-6111.

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XXI CONGRESS OF THE INTERNATIONAL ASSOCIATION OF LOGOPEDICS AND PHONIATRICS

The XXI Congress of the International Association of Logopedics and Phoniatrics will be held Aug 6-10, 1989, in Prague. For further information, contact the Secretariat, XXI Congress of the International Association of Logopedics and Phoniatrics, Czechoslovak Medical Society J. E. Purkyne, 120 26 Prague 2, Vitezneho unora 31, Czechoslovakia; telephone 29 41 41-4, telex 121 293.

Ann Otol Rhinol Laryngol 98:1989

PARTIAL LARYNGECTOMY AND RADIOTHERAPY FOR SUPRAGLOTTIC CANCER: A CONSERVATIVE APPROACH

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This is a retrospective study of 33 patients with supraglottic cancer treated with partial laryngectomy and moderate-dose radiotherapy at the University of Virginia from 1967 through 1986. All patients had a 2-year minimum follow-up. Ten patients received preoperative radiotherapy, and 23, postoperative radiotherapy. The 2-year adjusted survival rate was impressive at 97%. Local control at 2 years was 96%, including one patient who was salvaged with total laryngectomy. Control of the neck was 90% at 2 years. Ninety percent of the patients alive at 2 years had a functional voice. One patient required a completion laryngectomy for chronic aspiration. Conservation surgery with radiotherapy provides excellent survival as well as locoregional control rates with preservation of function in selected patients with supraglottic cancer. This approach may be especially helpful in the management of bulky T2 lesions as well as T4 lesions with minimal vallecula or base of tongue involvement.

KEY WORDS — conservation surgery, radiotherapy, supraglottic cancer.

INTRODUCTION

In several cancers such as carcinoma of the breast and soft tissue sarcomas of the extremities, there recently has been a trend toward using a combination of radiotherapy and conservative surgery in the hope of achieving the same cure rates as radical surgery alone without compromising function or cosmesis. Nowhere has this propensity been more prevalent than in the field of laryngology, where attempts to conserve the larynx invaded by cancer have been ongoing for many years. Ogura et al,[1] in particular, have explored the use of low-dose radiotherapy followed by conservation surgery in cancer of the supraglottis.

At the University of Virginia, the policy for treatment of supraglottic cancer has been radiotherapy with surgical salvage in reserve for T1, T2, and selected (where cords become mobile after 5,000 cGy) T3 cancers, with preoperative radiotherapy and total laryngectomy for nonradioresponsive T3 and all T4 lesions. A review of this experience demonstrated 3-year local control rates of 80% and 63% for T1 and T2 lesions, respectively.[2] Of particular interest, however, was a subpopulation of 12 patients treated at the discretion of the physician with partial laryngectomy and radiotherapy. In this group, who mainly had T2 lesions, the 3-year local control rate was 100% (including one salvage with total laryngectomy), with a survival rate of 83%. Ninety percent of the patients alive at 3 years had a functional larynx. This paper is an attempt to determine the validity of this observation from a review of our own continuing experience with conservation surgery and radiotherapy in cancers of the supraglottis. End points for analysis include survival, local control, nodal control, voice preservation, and complications.

PATIENTS

Thirty-six patients with squamous cell carcinoma of the supraglottis were treated with conservative surgery and radiotherapy at the University of Virginia from 1967 through 1986. The majority of patients received both surgical treatment and radiotherapy at this institution, while a few went elsewhere for either the surgical or radiotherapeutic part of their treatment. Thirty-three patients were followed for 2 or more years and form the basis of this retrospective analysis.

Patients ranged in age from 36 to 69 years, with 30% over age 60 (see Figure, A). The male to female ratio was 1.2 to 1. Ten patients developed second primary tumors, nine of which were in the aerodigestive tract. The differentiation of the supraglottic carcinoma was known in 27 patients, with 89% having moderately or poorly differentiated tumors. The site of the primary lesion within the supraglottis was the epiglottis in 70% of cases, the aryepiglottic fold in 18%, the false cord in 9%, and the arytenoid in 3%.

Distribution by T and N status is indicated in the Table. No patients presented with distant metastases. The T2 patients comprised a heterogeneous group, with 50% having three or more sites involved with tumor. Three of the four T3 patients had a fixed vocal cord, while the fourth patient had extension into the medial wall of the pyriform sinus.

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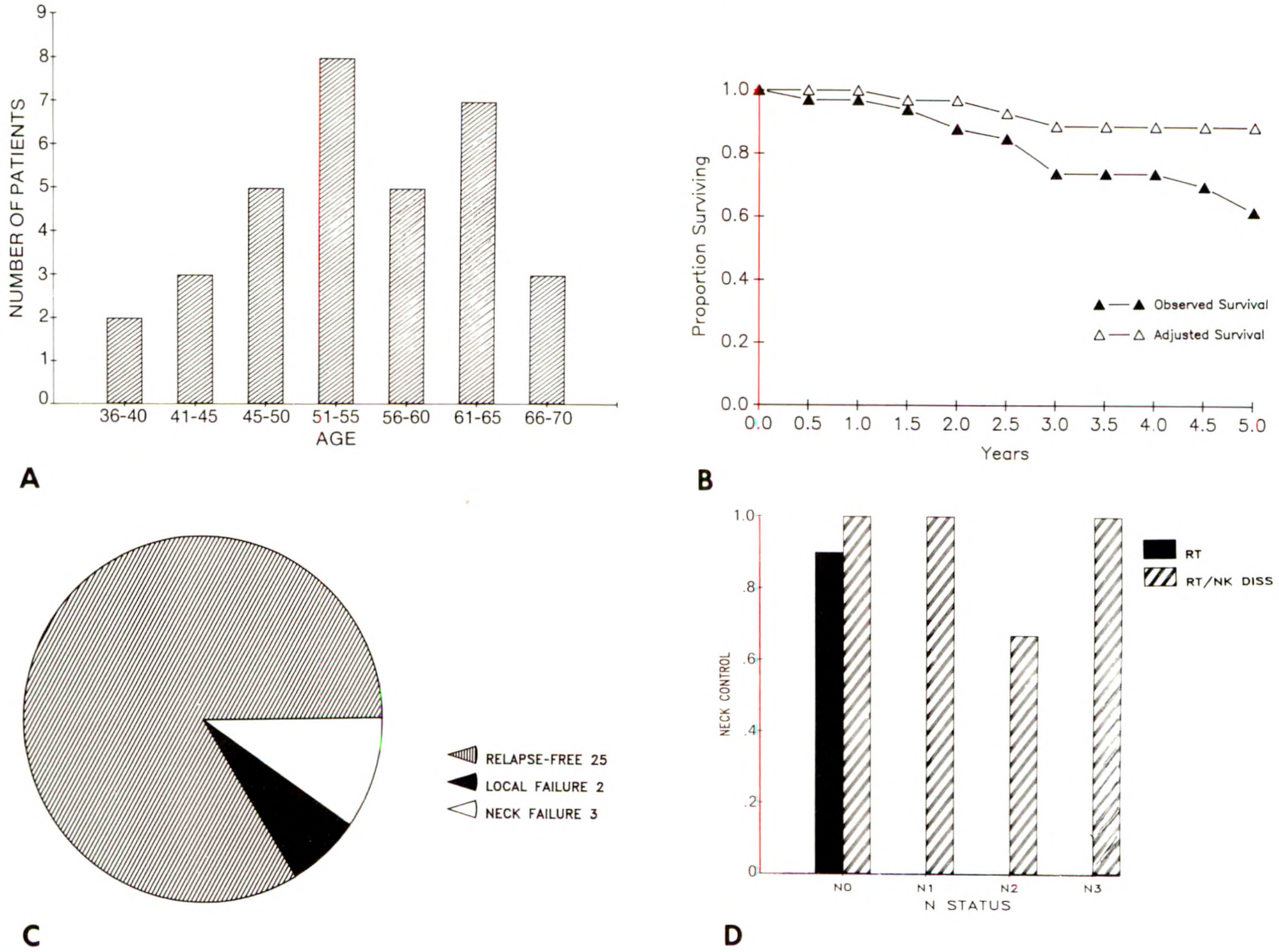

Study patients with squamous cell carcinoma of supraglottis. A) Age. B) Survival. C) Patterns of failure at 2 years. Numbers in key indicate numbers of patients. D) Neck control at 2 years by N status and treatment. RT — radiotherapy, NK DISS — neck dissection.

All of the T4 patients were assigned that status on the basis of vallecula or minimal base of tongue involvement. Two thirds of the patients had a clinically staged N0 neck at the time of diagnosis.

TREATMENT

Conservation surgery consisted of a supraglottic laryngectomy or extended supraglottic laryngectomy in 30 patients and of an extended vertical hemilaryngectomy in the T3 patients with a fixed vocal cord. Seventy percent of patients received postoperative radiotherapy, with the remainder receiving preoperative irradiation. The dose of preoperative radiotherapy was 5,000 cGy in all cases, while the postoperative dose tended to be higher, ranging from 5,000 to 6,100 cGy. The volume encompassed the primary tumor, base of tongue (when involved), and both sides of the neck. The treatment technique consisted of lateral or angled lateral neck fields, with larger field sizes used in the postoperative setting in order to cover the operative field adequately.

Survival data are derived from the entire group of 33 patients. Patients dying of intercurrent disease before 2 years who remained relapse-free are excluded from analysis of failure patterns.

RESULTS

Survival. Observed survival was 88% and 62% at 2 and 5 years, respectively, as illustrated in the Figure, B. Adjusted survival, reflecting deaths secondary to cancer, demonstrates a 2-year survival of 97%, which falls to 89% at 3 years and remains stable thereafter. Two patients died of cancer between 2 and 3 years, one at 28 months of lung metastases and the other of uncontrolled local recurrence (despite wide-field laryngectomy at 22 months) and lung metastases at 34 months. At 2 years, 29 patients remained alive with no evidence of supraglottic cancer, three had died of intercurrent disease, and one had died at 15 months with a neck recurrence. The last patient is excluded from the analysis of control at the primary site.

Locoregional Control. At 2 years, locoregional control was impressive, with only two patients relapsing with local disease and three with neck disease (see Figure, C).

DISTRIBUTION OF PATIENTS BY T AND N STATUS

	N0	N1	N2	N3	Total
T1	3	1			4
T2	11	1	2	2	16
T3	3	1			4
T4	5	3	1		9
Total	22	6	3	2	

The overall local control rate at 2 years was 93%. Including the results of salvage therapy, control rates rose to 96% (28/29). One hundred percent of T1 (4/4) and T2 (14/14) patients remained relapse-free at the primary site at 2 years. Eighty-two percent of T3 and T4 lesions were controlled by the initial treatment at 2 years (75% of T3 [3/4] and 86% of T4 [6/7]). Two patients failed locally: a T3 patient who relapsed in the glottis at 22 months, subsequently had a recurrence after an attempted salvage total laryngectomy, and died with disease at 34 months; and a T4 patient who failed in the base of tongue at 19 months and was salvaged with a total laryngectomy and partial glossectomy. Including salvage, then, the local control rate in the T3 and T4 lesions was 91%.

Information about the postoperative status of the margins was available in 21 of the patients receiving postoperative radiotherapy. Margins were clearly negative in ten patients and close in three patients, all of whom remained free of disease at the primary site. Margins were positive in eight patients; however, the addition of postoperative radiotherapy resulted in an 88% local control rate in these cases.

Control of the neck at 2 years was 90% (27/30). A neck dissection was performed in 30% (6/20) of the N0 patients alive at 2 years. Of the six patients with clinically staged N0 necks treated with neck dissection and postoperative radiotherapy, only one had nodes discovered pathologically. Overall local control in the N0 neck was 90% (18/20), with the two failures occurring among the 14 patients who received radiotherapy alone to the neck (see Figure, D). Both of these failures were salvaged with a neck dissection at the time of recurrence, yielding an overall neck control rate of 100% in N0 patients. All of the patients with palpable nodes were treated with a combination of neck dissection and radiotherapy, which is our institutional policy. Neck control in this group was also 90% (9/10), with one patient with N2 disease dying at 15 months with uncontrolled supraclavicular nodes and lung metastases. Distant metastases developed in two patients (both in the lung) after locoregional relapses.

Voice Conservation. Three patients required completion laryngectomies, two for recurrence and one, at 9 months, to relieve chronic aspiration problems. Ninety percent (26/29) of the patients alive at 2 years had functional voices. The majority of these patients had some degree of hoarseness; however, this did not impair communication.

Complications. Two patients developed serious complications following treatment that necessitated surgical correction. As mentioned, one patient required a total laryngectomy for chronic aspiration problems. Another patient developed a fistula following preoperative radiotherapy, supraglottic laryngectomy, and neck dissection that required surgical revision. Less morbid complications included a chylothorax following surgery, an aspiration problem that resolved without further treatment, one case of lymphedema in the neck, and a case of arytenoid edema that resolved after several months. The last two patients received 6,000 to 6,100 cGy of postoperative radiotherapy.

DISCUSSION

The optimal management of supraglottic cancer continues to be a matter of considerable debate between surgical and radiation oncologists. Clearly, the diversity in gross appearance, extent (often unaccounted for by T status), differentiation, and associated adenopathy found in cancers of the supraglottis necessitates a relatively complex formulation of treatment policy in order to achieve the best possible cure and conservation rates for the patient.

Harwood et al[3,4] are strong advocates of radiotherapy alone with surgical salvage in reserve for all cases of supraglottic cancer. With no selection factors for treatment, their overall 5-year survival was 41%, with 38% of patients dying of laryngeal cancer. Local control for stage I lesions was 78%, dropping to 67% for stage II. Nodal control in the N0 neck ranged from 82% to 89%. However, with the use of a larger treatment volume, there was a definite trend toward improved local (84%) and regional (96%) control for stage I and II disease in the last period of study (1975-1979). Approximately half of the advanced supraglottic lesions were controlled with the Princess Margaret Hospital treatment policy, with no advantage seen to upfront combined therapy. While these results of radiotherapy alone with surgery for salvage are in general quite good, the percentage of patients alive with voice preservation is not stated. As the surgical operation for recurrence is usually total laryngectomy, the majority of patients who were salvaged probably suffered the loss of their voice. Moreover, particularly in stage II disease, local control and survival have the potential for improvement.

Focusing specifically on T2 lesions, other radiotherapists report local control rates at 2 to 3 years of 59% to 89% with a policy of radiotherapy alone.[2,5-7] Voice preservation after radiation/surgical salvage for all patients treated in this way ranges from 43% to 83% in reported radiotherapy series.[2,6,7]

A number of reports in the literature expound the virtues of treatment of supraglottic cancer with conservative surgery. The goal here, of course, is the same as with radiotherapy as the primary modality: cure with preservation of function. The critical difference with regard to results is that by its very nature, conservative surgery can be carried out only in selected patients, although the contraindications for this type of surgery in supraglottic cancer are diminishing all the time with the improvement in surgical reconstructive techniques. Som[8] in 1970 reported an overall 2-year survival rate of 73% with a 1-year local recurrence rate of 7%; Bocca et al[9] in 1983 reported a 75% 5-year survival rate with a 14% incidence of local recurrence; Maceri et al[10] in 1985 reported an 80% 2-year disease-free survival with an overall local recurrence rate of 29%, and for stage II, a recurrence rate of 27%; and DeSanto[11] in 1985 reported a 76% survival rate and a 2% local recurrence rate overall as well as a 2% local recurrence rate for T2 lesions specifically. While local control rates with this approach are impressive, DeSanto[11] points out that failure in the neck remains a significant problem after unilateral dissection, particularly in patients with palpable neck disease at presentation, of whom 44% died of supraglottic cancer.

Fletcher et al[12] provide a convincing rationale for planned combined therapy in supraglottic cancer. In advanced lesions treated with a combination of total laryngectomy and radiotherapy, their local recurrence rates were only 14%. In the N2/N3 neck, radiotherapy reduced the neck recurrence rate after surgery alone from 36% to 10%. In the setting of conservative surgery, Ogura et al[1] and Marks et al[13] have been the major proponents of combination therapy with a regimen of 3,000 cGy preoperative radiotherapy followed by conservation surgery. They reported a 3-year actuarial survival rate of 67%. Three-year survival rates with no evidence of disease were 69%, 75%, 71%, and 63% for T1, T2, T3, and T4 lesions, respectively. The worst survival rate by site was 46% for patients with vallecula or base of tongue involvement. The voice was preserved in 71% of patients. As with surgery alone, the neck was the most common site of failure, occurring in 21% of patients. They concluded that 3,000 cGy was an inadequate dose for neck sterilization. Burstein and Calcaterra[14] reported a series of 41 patients treated with conservation surgery, 18 of whom received adjuvant radiotherapy with doses ranging from 5,000 to 6,000 cGy. Three of the four local recurrences and five of the seven neck failures occurred in patients who were treated with surgery only. Contralateral neck metastases predominated in the cases treated with surgery alone.

In the current selected group of patients treated with conservation surgery and moderate-dose radiotherapy, survival rates as well as locoregional control rates compare favorably with those of other reported series.[1-11,13,14] The particular sequence of radiotherapy in relation to surgical treatment did not appear to be a significant factor in this study. T2 and T4 lesions comprised the majority of this series, with the number of patients with T1 and T3 lesions too small to allow separate conclusions. All of the T2 lesions were controlled by the initial therapy, despite the bulky nature of half of the cases. Eighty-six percent of the T4 lesions remained relapse-free at the primary site, with the one failure salvaged by total laryngectomy and partial glossectomy. Ninety percent of patients alive at 2 years retained laryngeal speech. Of particular interest is the contribution that radiotherapy, in conjunction with surgery, made with regard to neck control. The control rate in the N0 neck treated with radiotherapy alone was 86%, which rose to 100% with combination treatment. The control rate in the N+ neck, with combined therapy used in all cases, was 90%.[15] The improvements in control and survival rates achieved with combined therapy were not offset by comparable gains in morbidity. In fact, complications remained minimal, with only one patient requiring a completion laryngectomy for chronic aspiration.

In conclusion, it is probably best to approach the management of supraglottic cancer with a policy that incorporates a variety of therapeutic strategies in order to optimize cure and laryngeal conservation. For T1 lesions, radiotherapy alone provides high cure rates. For exophytic, limited T2 lesions, radiotherapy with surgery in reserve appears reasonable. For T3 lesions, laryngeal function without compromise of cure rates may be achieved either with advances in conservation surgical techniques with radiotherapy as an adjunct or with a policy of preoperative irradiation to 5,000 cGy with a continuation to curative doses of radiation if the cords become mobile at that point.[16] For advanced T3 and T4 lesions, a combination of radiotherapy and total laryngectomy is still the mainstay of treatment. Conservation surgery with radiotherapy is currently of particular benefit in the management of large T2 lesions and lesions that are T4 on the basis of involvement of the vallecula or base of tongue.

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SIXTH WORLD CONGRESS IN ULTRASOUND

The Sixth World Congress in Ultrasound will be held September 1-6, 1991, in Copenhagen. For further information, contact Spadille Congress Service ApS, Sommervej 3, DK-3100 Hornbaek, Denmark; telephone 45 2 202496, telefax 45 2 200160.

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COCHLEAR MECHANICS AND OTOACOUSTIC EMISSIONS

The Second International Symposium on Cochlear Mechanics and Otoacoustic Emissions will be held March 9-11, 1989, in Rome. For further information, contact Centro Ricerche e Studi (CRS) Amplifon, Via Ripamonti 129, I-20141 Milano, Italy.

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IMPLANTATION OF A RECURRENT LARYNGEAL NERVE STIMULATOR FOR THE TREATMENT OF SPASTIC DYSPHONIA

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Spastic dysphonia, a rare speech disorder, is characterized by strained phonation with excessively adducted vocal cords. Recurrent laryngeal nerve section, botulinum toxin injection into the vocalis-thyroarytenoid muscle complex, and other techniques have been used to treat this disorder. We have used percutaneous electrical stimulation of the recurrent laryngeal nerve with good results. Previous dog studies demonstrated the relative safety of an implantable recurrent laryngeal nerve stimulator. In this study, we directly stimulated the recurrent laryngeal nerve and vagus nerve in a dog without change in cardiorespiratory status. A Medtronic peripheral nerve stimulator was implanted in a patient with abductor spastic dysphonia. The cuff electrode was positioned around the recurrent laryngeal nerve and stimulation resulted in improvement in her voice. Extensive cardiopulmonary monitoring did not reveal any adverse response to stimulation and there was no discomfort to the patient. On the basis of the good results of this preliminary study, further study with long-term follow-up is under way.

KEY WORDS — recurrent laryngeal nerve stimulator, spastic dysphonia, voice.

INTRODUCTION

Spastic dysphonia, a rare speech disorder, has recently sparked interest among otolaryngologists. Spastic dysphonia occurs in either an abductor or adductor variety. The classic adductor form is characterized by high-pitched staccato phonation with voice arrests and various degrees of facial grimacing. These patients have hyperadduction of the true and false vocal cords with simultaneous elevation of the larynx.[1] The abductor form of spastic dysphonia is characterized by a whisper or weak, breathy phonation, particularly at voicing onsets.

The cause and pathophysiology of spastic dysphonia remain unknown despite many successfully treated cases. Most investigators agree that spastic dysphonia has an important psychogenic component. Others focus on the neurologic aspects of the disease and implicate the extrapyramidal tracts as the possible site of lesion.[1] After studying electromyographic findings in patients with spastic dysphonia, Blitzer et al[2] classified most patients with spastic dysphonia as having a type of dystonia presenting focally or in conjunction with other types of dystonias.

The success of all current treatments for spastic dysphonia is variable and appears to be related to patient selection. Patients with spastic dysphonia that has an obvious psychogenic component benefit most from psychotherapy, biofeedback, or voice-speech therapy.[3] Patients with adductor spastic dysphonia of true neurologic cause (laryngeal hyperstimulation from extrapyramidal tracts) are more likely to benefit from treatment that alters motor transmission to the recurrent laryngeal nerve. Recurrent laryngeal nerve section alters motor activity of the ipsilateral vocal cord. A success rate of 97% 1 month postoperatively was reported with recurrent laryngeal nerve section.[3,4] However, the success rate decreased to 36% after 3 years' follow-up because of gradual overadduction of the contralateral vocal fold against the paralyzed vocal fold.[3]

Blitzer et al[2] reported dramatic improvement in five spastic dysphonia patients treated with botulinum toxin. The botulinum toxin was injected percutaneously through the cricothyroid membrane into the vocalis-thyroarytenoid muscle complex under electromyographic localization. The technique of electromyographic localization is difficult and time-consuming. Botulinum toxin takes effect within 48 to 72 hours and lasts from 3 to 9 months.[4] Disadvantages are the inconsistent effect and duration of action of the botulinum toxin. Patients often require repeat injections, bilateral injections, and increased dosages to achieve the desired effect. Excessive or inexperienced use of botulinum toxin may lead to bilateral vocal cord paralysis and airway compromise. Furthermore, the long-term effects of botulinum toxin on the larynx are not yet known.

Friedman et al[5] reported on percutaneous electrical stimulation of the recurrent laryngeal nerve for temporary treatment of spastic dysphonia. All of the patients who underwent percutaneous stimulation had some degree of improvement, with the majority having dramatic improvement. A peripheral

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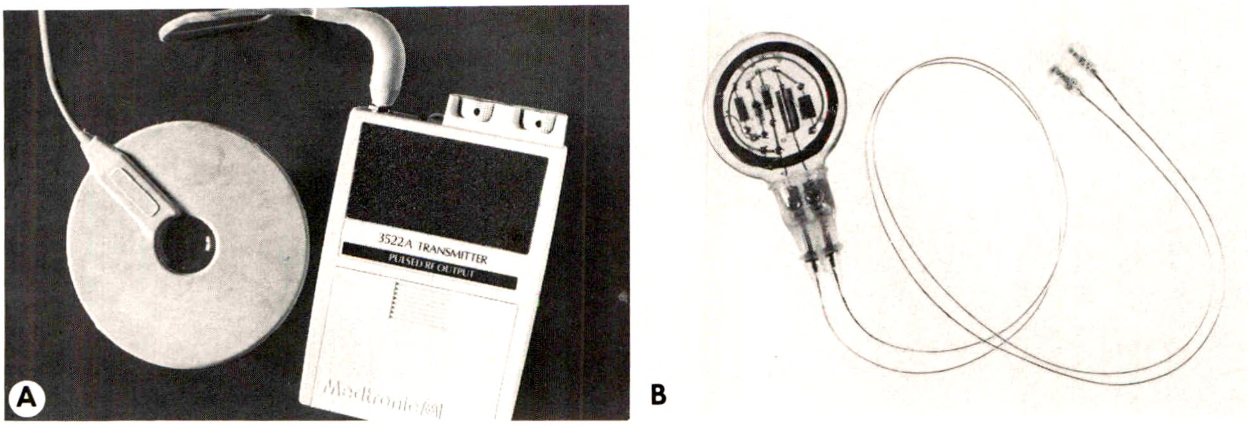

Fig 1. Apparatus. A) Medtronic model 3522A peripheral nerve stimulator. Transmitter and external antenna. B) Implantable receiver and 2-mm cuff electrode.

nerve stimulator with a cuff electrode positioned around the recurrent laryngeal nerve was implanted in a dog to study its effect on cardiorespiratory status and the long-term effect on the nerve. There was no alteration in the cardiorespiratory status of the dog during direct stimulation of the recurrent laryngeal nerve. The cuff electrode caused some perineural fibrosis, with only slight endoneural fibrosis. Vocal cord function was normal.

EXPERIMENTAL STUDY

A single mongrel dog underwent direct electrical stimulation of both the recurrent laryngeal nerve and the vagus nerve using a bipolar cuff electrode and peripheral nerve stimulator (Medtronic, Inc, Minneapolis). Anesthesia was induced with intravenous sodium pentobarbital (30 mg/kg), and the dog was placed in the supine position with the neck shaved, prepared, and draped. A midline incision was made at the level of the cricoid cartilage, and the strap muscles were separated in the midline. The right recurrent laryngeal and vagus nerves were identified and carefully dissected from the surrounding soft tissue.

A bipolar cuff electrode was placed around the recurrent laryngeal nerve, which was stimulated at settings from 2 to 10 mA, with frequencies from 10 to 100 Hz and a pulse width of 0.5 ms. The dog's cardiac and respiratory status were monitored continuously. There were no cardiorespiratory changes at settings from 2 to 7 mA and frequencies from 10 to 70 Hz. Current greater than 7 mA resulted in adjacent muscle stimulation and a slight increase in heart rate. Frequencies greater than 70 Hz resulted in complete adduction and glottic closure. Direct electrical stimulation of the vagus nerve at settings from 2 to 10 mA and frequencies of 10 to 100 Hz did not result in any change in cardiorespiratory status.

CLINICAL STUDY

The primary purpose of this portion of the study was to evaluate the safety of an implanted recurrent laryngeal nerve stimulator for the treatment of spastic dysphonia. Of major concern was the effect of electrical stimulation of the recurrent laryngeal nerve on cardiorespiratory status, because of the close proximity to the vagus nerve.

Patient Selection. Diagnosis of spastic dysphonia was based on examination by two otolaryngologists and two speech pathologists experienced in the evaluation of patients with spastic dysphonia. Spastic dysphonia patients were characterized as having predominately an adductor or abductor variety of the disease. We required patients to undergo at least 12 months of psychotherapy and speech therapy prior to being considered for implantation. Percutaneous electrical stimulation of the recurrent laryngeal nerve was used to predict the benefit of the implantable device. If improvement on percutaneous stimulation of the recurrent laryngeal nerve occurred, the patient was considered a candidate for implantation of the recurrent laryngeal nerve stimulator. A preoperative videotape documented pretreatment status of the disease.

Patient Evaluation. Postoperative voice was scored subjectively by two otolaryngologists and two speech pathologists using a scale from 1 to 4 (1 = worse, 2 = no change, 3 = improvement, 4 = marked improvement). Patients were evaluated before and after percutaneous stimulation and nerve stimulator implantation.

Nerve Stimulator. The transmitter was a Medtronic peripheral nerve stimulator (model 3522A, Neuro Division, Medtronic, Inc) with an external antenna (Fig 1A). The implantable receiver and 2-mm bipolar cuff electrode (Fig 1B) were custom-made to accommodate the recurrent laryngeal nerve (Custom Med Laboratories, Inc, New Durham, NH). The implantable device was approved for use by the Food and Drug Administration Center for Devices and Radiologic Health.

Technique for Implantation. The neck and chest are prepared to the nipple. A gently curved hori-

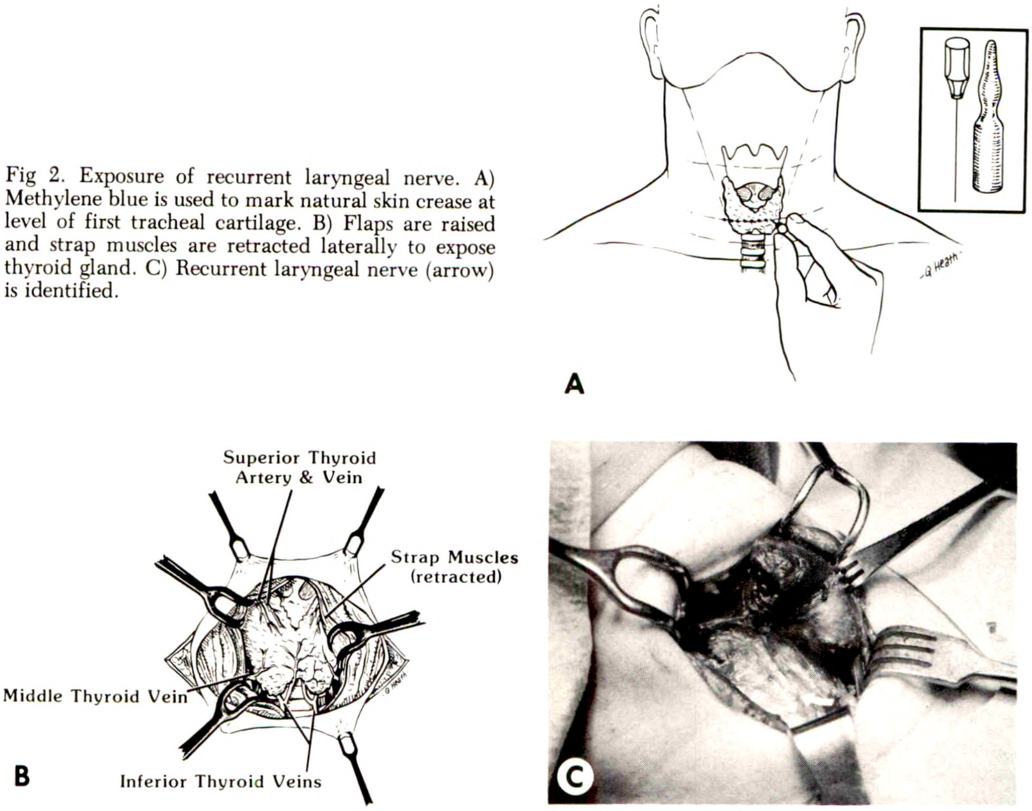

Fig 2. Exposure of recurrent laryngeal nerve. A) Methylene blue is used to mark natural skin crease at level of first tracheal cartilage. B) Flaps are raised and strap muscles are retracted laterally to expose thyroid gland. C) Recurrent laryngeal nerve (arrow) is identified.

zontal (collar) incision is made in a natural skin crease at the level of the first tracheal cartilage (Fig 2A). Flaps are raised in the subplatysmal plane with the superior flap extending to the hyoid bone and the inferior flap to the clavicle. Strap muscles are separated in the midline and retracted laterally to expose the right thyroid lobe (Fig 2B). The thyroid gland should be palpated for masses. After the inferior thyroid vein is dissected, the inferior thyroid artery is identified between the trachea and carotid artery and followed medially behind the gland, where it crosses the recurrent laryngeal nerve. After the nerve is identified (Fig 2C), the inferior thyroid artery can be ligated close to the thyroid gland (only if necessary) to expose the nerve. The inferior parathyroid gland must be preserved.

The lower pole of the thyroid can be dissected medially to expose most of the nerve. The recurrent laryngeal nerve should be dissected carefully from surrounding connective tissue to free a 2-cm segment of nerve near the lower pole of the thyroid lobe.

A subcutaneous pocket is dissected just lateral to the right midclavicular line about 4 cm above the nipple. In women, the pocket may need to be positioned further inferolaterally to accommodate different styles of dress. The pocket is formed by tunnelling from the neck incision over the clavicle and through the subdermal fat over the pectoralis major muscle, and should be about 4 x 4 cm in size. Long Richter scissors or a long clamp can be used to make the tunnel and pocket. Blunt dissection also can be used if meticulous hemostasis is maintained and adequate visualization of the tunnel is achieved with a Kentwood Ramus retractor with fiberoptic illumination.

The cuff electrode is positioned carefully around the recurrent laryngeal nerve and the electrode is sutured to surrounding connective tissue for stability (Fig 3A,B). The cuff electrode should surround the nerve without compressing it and the cuff electrode itself is sutured to surrounding soft tissue to prevent rotation or kinking of the nerve. The subcutaneous pocket is examined to verify the final location of the receiver (Fig 3C).

The receiver is passed through the tunnel into the subdermal pocket with care taken not to twist the leads (Fig 3D,E). A suction drain is inserted in the neck and the skin incision is closed in two layers. A 1-week course of prophylactic oral antibiotics is administered.

Postoperative Monitoring. Postoperatively, the patients were kept in an intensive care unit for 48 hours on continuous cardiac and respiratory monitoring. During the 48 hours following the intensive care monitoring, a Holter monitor was used. The nerve was stimulated continually at settings of 2 to 9 mA, frequencies ranging from 10 to 100 Hz, and a pulse width of 0.5 ms.

Evaluation of Phonation. By the method previously described, a patient's response to stimulation

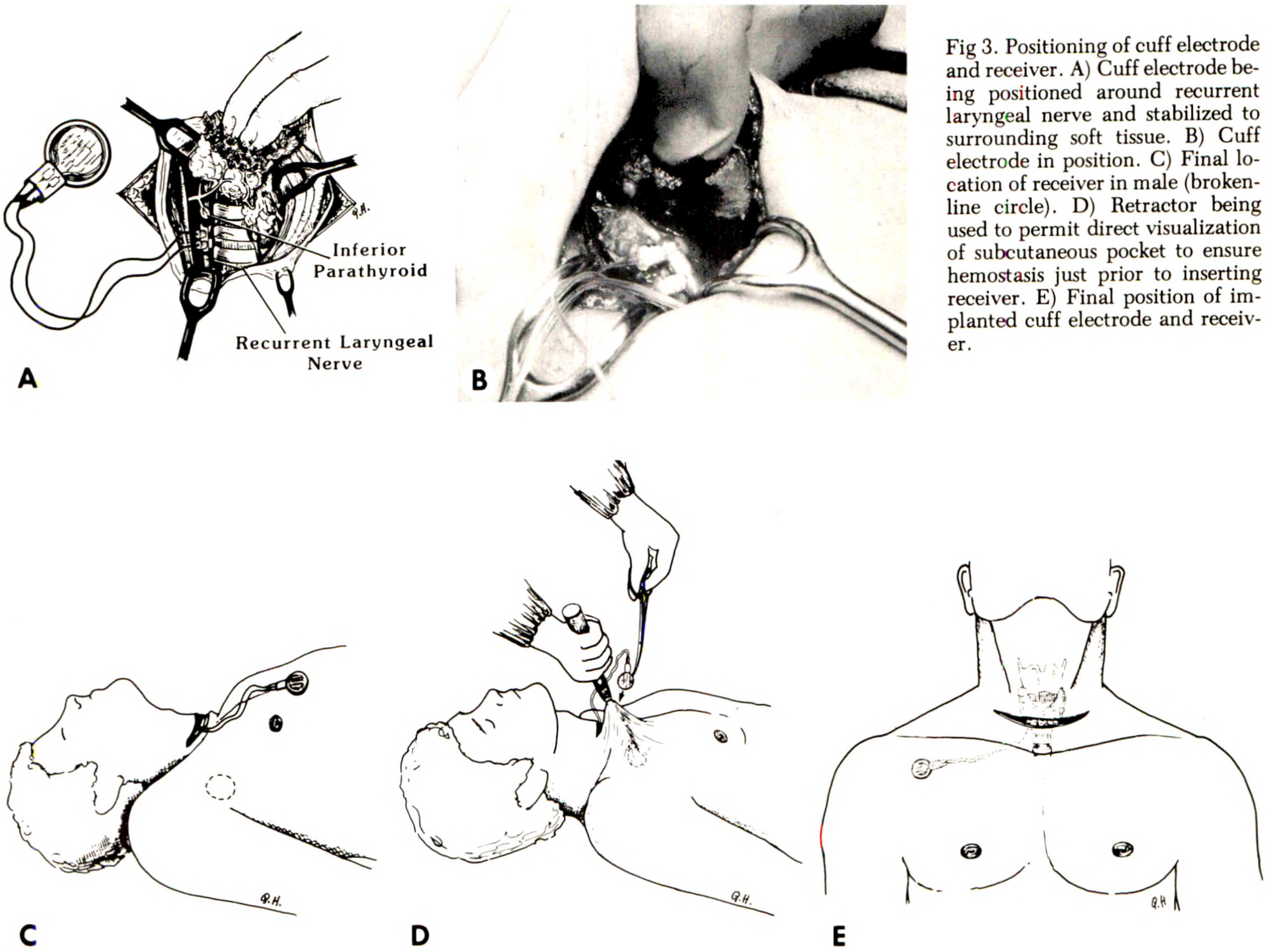

Fig 3. Positioning of cuff electrode and receiver. A) Cuff electrode being positioned around recurrent laryngeal nerve and stabilized to surrounding soft tissue. B) Cuff electrode in position. C) Final location of receiver in male (broken-line circle). D) Retractor being used to permit direct visualization of subcutaneous pocket to ensure hemostasis just prior to inserting receiver. E) Final position of implanted cuff electrode and receiver.

was assessed. Flexible fiberoptic laryngoscopy was used to evaluate cord position during stimulation.

CASE REPORT

A 42-year-old woman had an 8-year history of abductor spastic dysphonia. She underwent psychotherapy and speech therapy for 5 years with no voice improvement. She had no prior neck operations or history of psychiatric disorder. On flexible fiberoptic laryngoscopy, her larynx was normal in appearance, except during phonation. Her voice was weak, particularly on voice onsets, but she had no facial grimacing. On percutaneous needle electrical stimulation of her right recurrent laryngeal nerve, her evaluation score was 2.6 (improvement). She underwent implantation of the recurrent laryngeal nerve stimulator and had an uneventful postoperative course.

Postoperative Monitoring. Throughout the 4-day postoperative monitoring period, there was no arrhythmia or change in respiratory status. The patient had no symptoms of fluttering sensation in her chest or chest pain. An electrocardiogram taken prior to hospital discharge was unchanged from the preoperative tracing.

There was no discernible change in respiratory rate or cardiac rhythm at 2 to 9 mA, frequencies ranging from 10 to 100 Hz, and pulse width of 0.5 ms. However, at 6 mA or greater the patient did notice a mild twitching sensation in her neck that was neither seen nor palpated by observers. At frequencies over 50 Hz, the patient noticed a tight sensation in her larynx but had no shortness of breath.

Phonation. Postoperatively the patient considered her speech to be improved. She received a compiled grade of 3 (improvement). At effective stimulation levels of 2 to 4 mA and 10 to 25 Hz, her cardiorespiratory status was unaltered, and she had no discomfort. On flexible fiberoptic laryngoscopy, it was evident that the vocal cord could be controlled by varying the stimulator frequency. Less than 5 Hz caused no visible change in the larynx, but frequencies of 10 to 25 Hz caused some movement of the vocal cord toward the midline. Twenty-five to 50 Hz caused a spasm in the right hemilarynx but no shortness of breath. Increasing the current over 2 mA had no effect on the larynx that could be discerned through the fiberoptic laryngoscope.

DISCUSSION

Accurate diagnosis, patient selection, and understanding of the limitations of different treatment modalities are the keys to successful management of spastic dysphonia.

Recurrent laryngeal nerve section leaves the patient with a paralyzed vocal cord and a high probability of recurrent symptoms.[3] There are theoretic advantages of recurrent laryngeal nerve stimulation over nerve section. An implanted recurrent laryngeal nerve stimulator should be able to be removed without irreversible damage to the nerve if the patient and the physician are not satisfied with the response. A severed nerve cannot be repaired with functional restoration.

The results of our study verify the relative safety of an implantable device. The absence of cardiorespiratory changes with therapeutic levels of stimulation supports the results of our studies in dogs.[5] Despite these encouraging results, all patients with implants should be under intensive care monitoring for the first 48 hours, followed by Holter monitoring for an additional 48 hours.

The degree of improvement in phonation after recurrent laryngeal nerve section, botulinum toxin injection, and electrical stimulation of the recurrent laryngeal nerve is greatest in patients with adductor spastic dysphonia.[3-5] Our patient had abductor spastic dysphonia, yet her response to electrical stimulation was impressive and paralleled our results with percutaneous stimulation.

Long-term clinical application of this treatment modality requires much further study with long-term follow-up, which is currently under way. Although preliminary results are good, the treatment remains experimental at present.

ACKNOWLEDGMENTS — The authors thank Roger E. Avery of Custom Med Laboratories, Inc, for providing the electrode and technical assistance, Chet Childs of the University of Illinois Eye and Ear Infirmary for audiovisual management, Terri Strorigl for editorial assistance, and Quinn Heath for the illustrations.

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ASYMMETRY OF THE LARYNGEAL FRAMEWORK: A MORPHOLOGIC STUDY OF CADAVER LARYNGES

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Asymmetry of the laryngeal framework was investigated with 50 excised human larynges, ten from newborns (five males, five females), 20 from adults in their 20s (ten males, ten females), and 20 from adults in their 50s (ten males, ten females). All adults were right-handed. The results are summarized as follows. 1) The laryngeal framework was asymmetric to a greater or lesser extent in all larynges. 2) The degree of asymmetry did not differ among different age groups or between sexes. 3) In newborns, there was no directional preponderance in asymmetry. 4) In older adults, there was a directional preponderance in asymmetry. The right thyroid lamina tended to tilt laterally whereas the left lamina showed a tendency to tilt medially. The right cricoarytenoid joint tended to be located slightly more laterally, posteriorly, and inferiorly than the left joint. The longitudinal axis of the thyroid cartilage was inclined to shift to the right posteriorly against the axis of the cricoid cartilage. The thyroid cartilage as a whole tended to tilt to the right against the cricoid cartilage. 5) There must be some compensatory mechanisms for the asymmetric framework to keep the vocal fold edges relatively symmetric.

KEY WORDS — asymmetry, laryngeal framework, normal variation.

It is well known that many people have an asymmetric larynx. From a clinical point of view, it is extremely important to determine whether a given asymmetry of the larynx is within the range of normal variations or is a disordered condition. Honjo et al[1] attributed a clinically observed group of asymmetric false folds to an asymmetric thyroid cartilage that was a normal variation. Asymmetries of the cartilaginous framework of the larynx have been described in some reports.[2-6] However, detailed analyses of asymmetry are few. The purpose of this study is to investigate asymmetry of the laryngeal framework in a normal population of different age groups.

METHODS

A total of 50 fresh Japanese cadaver larynges obtained at the time of autopsy were included in this study. Of the 50 larynges, ten were from newborns (five males, five females), 20 were from adults in their 20s (ten males, ten females), and 20 were from adults in their 50s (ten males, ten females). All adults were right-handed. None of the larynges had any sign of disease and their structures appeared normal.

Following removal of the soft tissue, measurements of 17 selected dimensions and four selected angles of the laryngeal framework were taken.

Thyroid Cartilage (Fig 1). The items selected for the measurements of the thyroid cartilage were width of the thyroid lamina at the upper edge (DTU), width of the thyroid lamina at the level of the anterior commissure (DTA), width of the thyroid lamina at the lower edge (DTL), height of the thyroid lamina at the inferior tuberculum (HTM),

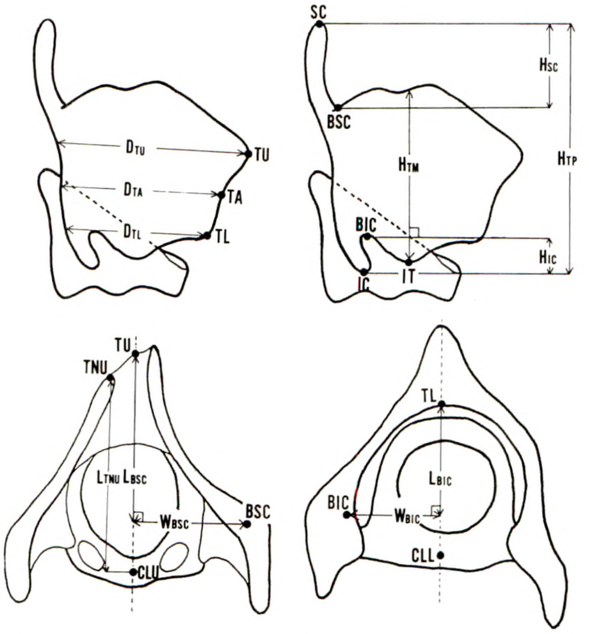

Fig 1. Measurements for thyroid cartilage. See Methods for definition of additional abbreviations. TU — upper edge at anterior midline, TA — level of anterior commissure at anterior midline, TL — lower edge at anterior midline, IT — inferior tuberculum, SC — tip of superior cornu, BSC — base of superior cornu, IC — tip of inferior cornu, BIC — base of inferior cornu, TNU — tip of superior thyroid incisure, CLU — midpoint of cricoid lamina at upper edge, CLL — midpoint of cricoid lamina at lower edge.

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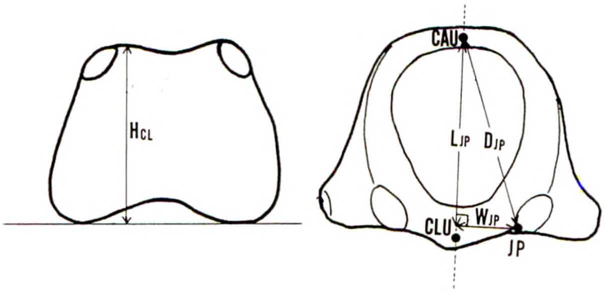

Fig 2. Measurements for cricoid cartilage. See Methods for definition of additional abbreviations. CAU — midpoint of cricoid arch at upper edge, CLU — midpoint of cricoid lamina at upper edge, JP — posterior end of facies articularis arytenoidea.

distance between the tips of the superior and inferior cornua (HTP), length of the superior cornu (HSC), length of the inferior cornu (HIC), distance of the tip of the superior thyroid incisure from the posterior plane at the cricoid lamina (LTNU)* (*indicates items measured on photographs), distance of the base of the superior cornu from the midline of the larynx (WBSC)* (the midline was defined as the line between the anterior midpoint of the thyroid cartilage and the posterior midpoint of the cricoid lamina), distance of the base of the superior cornu from the frontal plane at the anterior edge (LBSC)*, distance of the base of the inferior cornu from the midline of the larynx (WBIC)*, and distance of the base of the inferior cornu from the frontal plane at the anterior edge (LBIC)*.

Cricoid Cartilage (Fig 2). The items selected for measurement of the cricoid cartilage were height of the cricoid lamina at the highest location (HCL), distance of the posterior end of the facies articularis arytenoidea (JP) from the midsagittal plane of the cricoid cartilage (WJP)*, distance of JP from the frontal plane at the anterior edge (LJP)*, and distance of JP from the anterior midpoint of the cricoid arch (DJP)*.

Cricothyroid Complex (Fig 3). The angle of the longitudinal axis of the thyroid cartilage against that of the cricoid cartilage at the upper edge (ACTU)* was measured. The longitudinal axis of the thyroid cartilage was defined as the line between the midpoint of the upper thyroid edge (TU) and the midpoint between the bilateral bases of the superior cornua (BSC). The longitudinal axis of the cricoid cartilage was defined as the line between the midpoint of the cricoid arch (CAU) and that of the cricoid lamina (CLU).

The angle of the longitudinal axis of the thyroid cartilage against that of the cricoid cartilage at the lower edge (ACTL)* was measured. The longitudinal axis of the thyroid cartilage was defined as the line between the midpoint of the lower thyroid edge (TL) and the midpoint between the bilateral bases of the inferior cornua (BIC). The longitudinal axis of the cricoid cartilage was defined as the line between the midpoint of the cricoid arch (CAL) and that of the cricoid lamina (CLL).

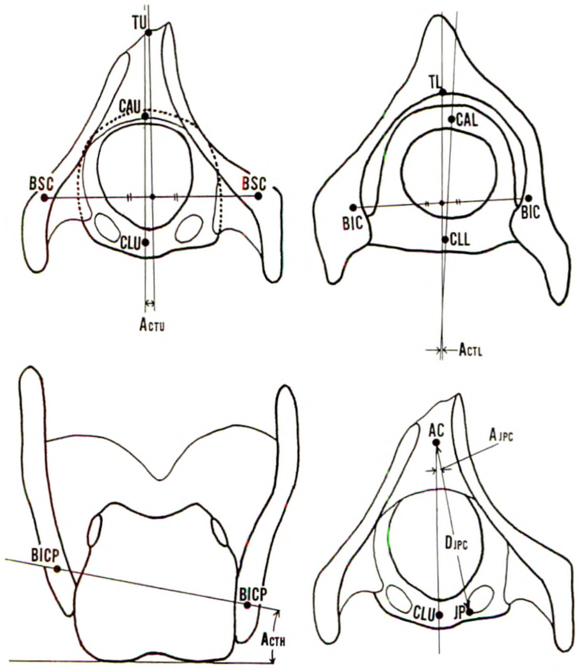

Fig 3. Measurements for cricothyroid complex. See Methods for definition of additional abbreviations. TU — upper edge of thyroid cartilage at anterior midline, CAU — midpoint of cricoid arch at upper edge, CLU — midpoint of cricoid lamina at upper edge, BSC — base of superior cornu, TL — lower edge of thyroid cartilage at anterior midline, CAL — midpoint of cricoid arch at lower edge, CLL — midpoint of cricoid lamina at lower edge, BIC — base of inferior cornu, BICP — posterior aspect of base of inferior cornu, AC — anterior commissure, JP — posterior end of facies articularis arytenoidea.

The angle of the thyroid cartilage against the cricoid cartilage in the frontal plane (ACTH)* was defined as the angle of the line between the bilateral bases of the inferior cornua against the horizontal plane at the lower edge of the cricoid cartilage.

The distance between the anterior commissure (AC) and the JP of the cricoid cartilage (DJPC)* was measured.

The angle of the line between the anterior commissure and the JP of the cricoid cartilage against the midsagittal plane of the larynx (AJPC)* was measured. This line was close to the location of the vocal fold edge. The midsagittal plane of the larynx was defined as the plane that contained the AC and the midpoint of the cricoid lamina.

The points of interest were marked with India ink in order to facilitate exact measurements. The measurements were conducted in one of the following two ways: a direct measurement with specimens by means of slide calipers and measurement on photographs. Measurements were repeated three times for each item and the median value was employed. Measurements were accurate to 0.04 mm.

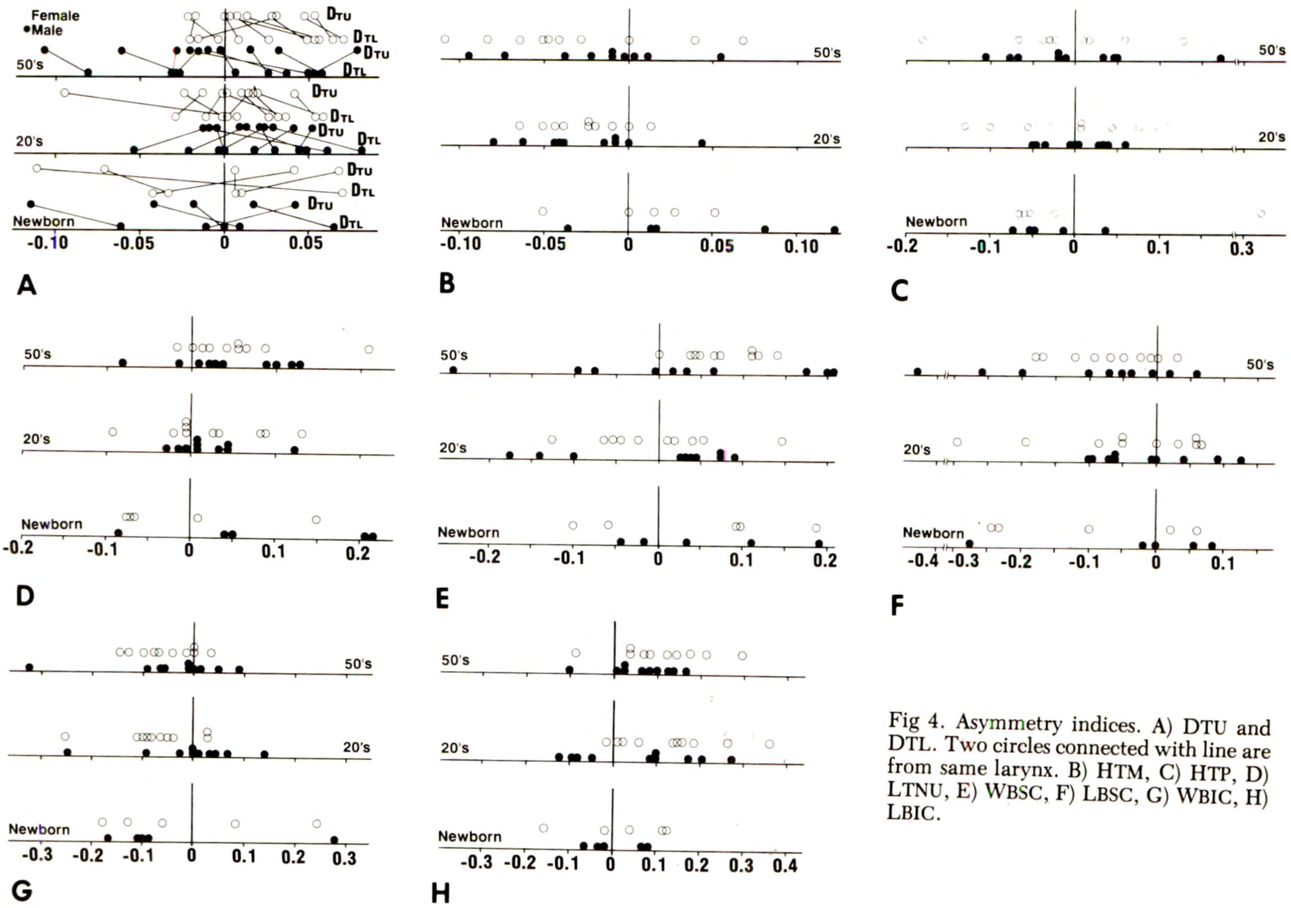

Fig 4. Asymmetry indices. A) DTU and DTL. Two circles connected with line are from same larynx. B) HTM, C) HTP, D) LTNU, E) WBSC, F) LBSC, G) WBIC, H) LBIC.

For the dimensions investigated, the degree of asymmetry was represented by the asymmetry index (AI), which was defined as

$$AI = \frac{R - L}{(R + L)/2}$$

where R was the measured value for the right side and L was that of the left side. When the value is the same for both sides, the AI becomes 0. When the measured value for the right side is greater than that of the left side, the AI assumes a positive value, whereas it presents a negative value when the measured value is greater on the left side. For the angles studied, the measured values were compared directly and discussed.

RESULTS

Since the purpose of this paper is to investigate asymmetry of the laryngeal framework, only those data that are related directly to asymmetry will be presented. The measured values for each item will be reported elsewhere.

All dimensions and angles investigated differed to a greater or lesser extent between the right and left sides in the majority of the larynges in all three age groups. In other words, all larynges were more or less asymmetric. The degree of asymmetry did not differ systematically among the age groups or between the sexes. In the following paragraphs, we will focus chiefly on directional preponderance in asymmetry.

Thyroid Cartilage. Figure 4A shows the AI for the width of the thyroid lamina (DTU and DTL). The distribution of the AI was also similar for DTA. The width was greater on the right side than on the left side (positive AI values) in some larynges, whereas it was greater on the left (negative AI values) in the others. In other words, there was no directional preponderance in asymmetry in any age group. The absolute value of the AI was less than 0.1 in almost all cases. The AI value of 0.1 implies that the right-left difference in measured value is 10% of the mean of the value for the right side added to that for the left side.

The height of the thyroid lamina (HTM) was greater on the right side than on the left side (positive AI values) in the majority of the newborns, whereas HTM was greater on the left (negative AI values) in the majority of the adult larynges (Fig 4B). The absolute value of the AI was less than 0.1 in all but two cases.

The distance between the tip of the superior cornu and that of the inferior cornu (HTP) was slightly greater on the left (negative AI values) in the majority of newborns, whereas in adults, the AI assumed

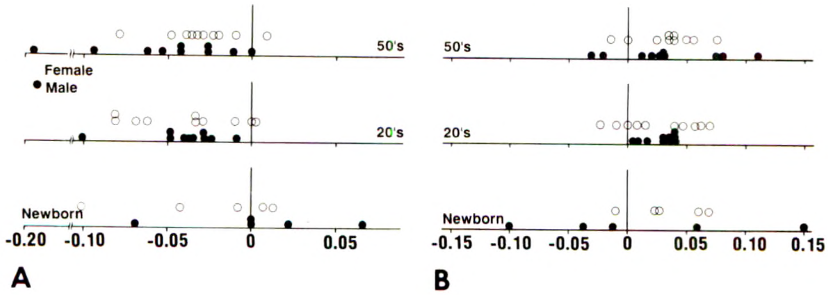

Fig 5. Asymmetry indices. A) Height of cricoid lamina at highest location. B) Distance of posterior end of facies articularis arytenoidea from anterior midpoint of cricoid arch.

both negative and positive values, demonstrating no directional preponderance in asymmetry (Fig 4C). The absolute value of the AI was less than 0.1 in many larynges, but in some it was greater than 0.1. The superior and inferior cornua of several larynges showed marked right-left differences in length, presenting absolute values of the AI of greater than 0.2. Asymmetry for the superior and inferior cornua, however, did not show any directional preponderance in any age groups.

For the distance of the tip of the superior thyroid incisure from the posterior plane at the cricoid lamina (LTNU), the AI assumed positive values in most adults in their 50s (Fig 4D). In other words, the right tip of the superior thyroid incisure was located anterior to the left tip. The same tendency was noted also for the adults in their 20s, but to a lesser extent. The anteroposterior distance between the bilateral tips varied. In some larynges, the right tip was located anterior to the left by 3 mm or more.

The location of the bases of the superior and inferior cornua presented a unique pattern of asymmetry in the adults, especially in the oldest age group (Fig 4E-H). In the 50s age group, the AI for the distance of the base of the superior cornu from the midline (WBSC) presented positive values (Fig 4E), whereas the AI for the distance from the anterior frontal plane (LBSC) assumed negative values (Fig 4F) in most larynges. This indicated that the upper edge of the right thyroid lamina tended to be located more anterolaterally than that of the left lamina. This tendency was not very marked in the adults in their 20s or in the newborns. The absolute value of the AI exceeded 0.1 in many larynges, indicating the existence of a considerable degree of asymmetry.

As for the base of the inferior cornu, the AI for the distance from the midline (WBIC) showed negative values (Fig 4G), whereas the AI for the distance from the anterior frontal plane (LBIC) assumed pos-

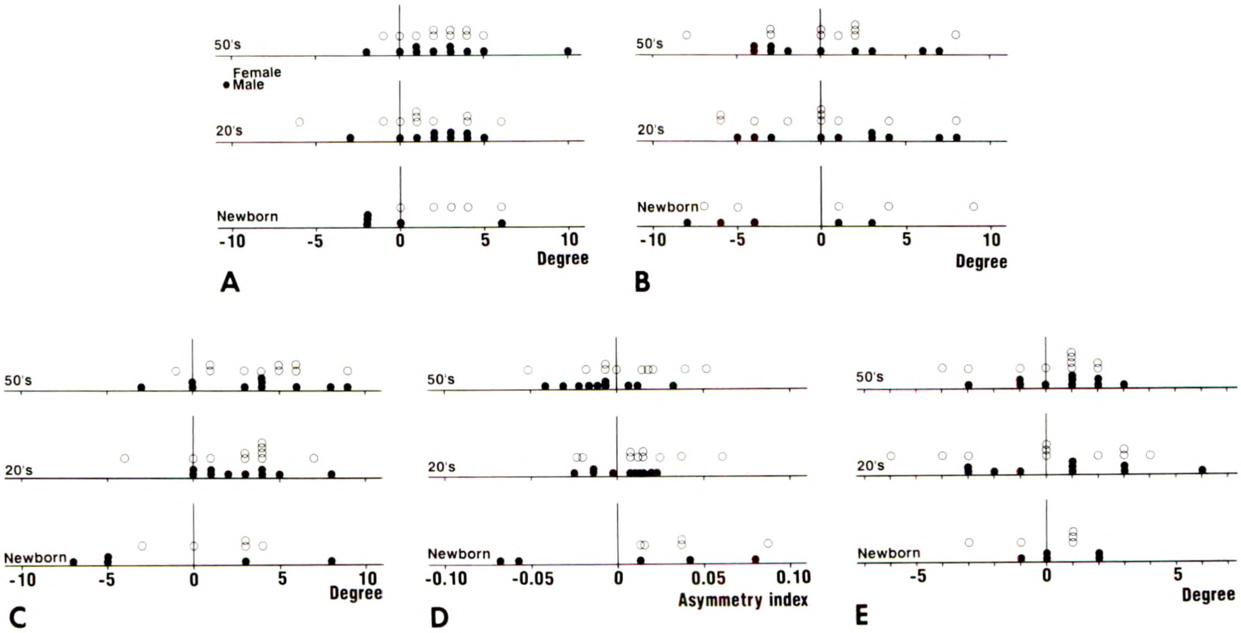

Fig 6. Results for cricothyroid complex. A) Value in degrees of ACTU, B) value in degrees of ACTL, C) value in degrees of ACTH, D) asymmetry index for DJPC, E) differences in degrees in AJPC between two sides. For ACTU and ACTL, positive values indicate that axis of thyroid cartilage is shifted to right posteriorly against axis of cricoid cartilage by degree indicated, whereas negative values indicate that thyroid axis is shifted to left posteriorly. For ACTH, positive values indicate that thyroid cartilage is tilted to right against cricoid cartilage, whereas negative values imply that thyroid cartilage is inclined to left. Values for AJPC were obtained by subtracting left AJPC value from right AJPC value. Therefore, positive values imply that angle is greater on right side than on left side.

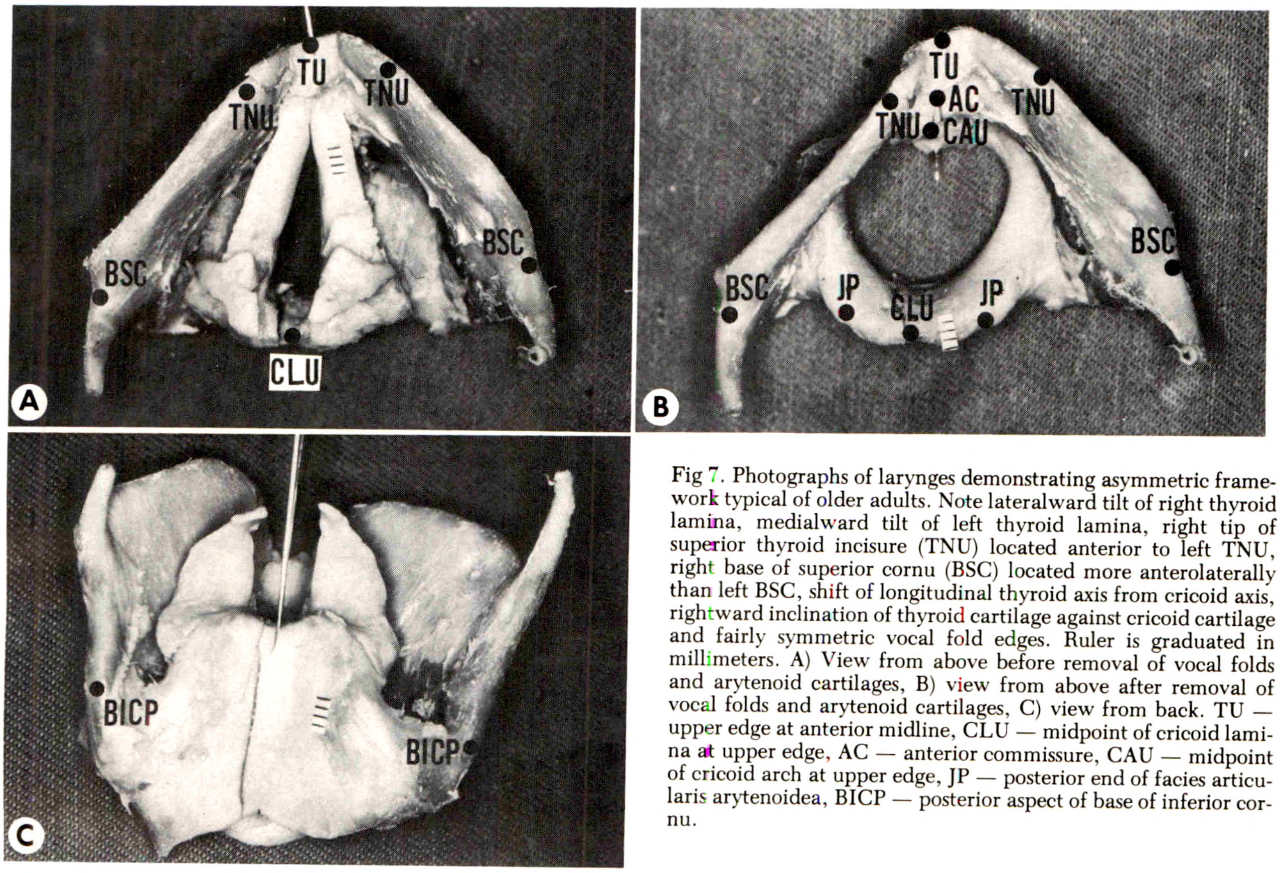

Fig 7. Photographs of larynges demonstrating asymmetric framework typical of older adults. Note lateralward tilt of right thyroid lamina, medialward tilt of left thyroid lamina, right tip of superior thyroid incisure (TNU) located anterior to left TNU, right base of superior cornu (BSC) located more anterolaterally than left BSC, shift of longitudinal thyroid axis from cricoid axis, rightward inclination of thyroid cartilage against cricoid cartilage and fairly symmetric vocal fold edges. Ruler is graduated in millimeters. A) View from above before removal of vocal folds and arytenoid cartilages, B) view from above after removal of vocal folds and arytenoid cartilages, C) view from back. TU — upper edge at anterior midline, CLU — midpoint of cricoid lamina at upper edge, AC — anterior commissure, CAU — midpoint of cricoid arch at upper edge, JP — posterior end of facies articularis arytenoidea, BICP — posterior aspect of base of inferior cornu.

itive values (Fig 4H) in the majority of the adults in their 50s. In other words, the lower edge of the right thyroid lamina was located more posteromedially than that of the left lamina. This phenomenon was noted also in the adults in their 20s, but with more exceptions. Again, the absolute value of the AI was greater than 0.1 in many cases, indicating the existence of a marked asymmetry.

The results shown in Fig 4D-H as a whole indicate that with aging, the right thyroid lamina tends to tilt laterally while the left lamina tends to tilt medially.

Cricoid Cartilage. The AI for the height of the cricoid lamina (HCL) presented negative values in almost all adults of both age groups (Fig 5A). In other words, the cricoid lamina was higher on the left side than on the right side. This phenomenon was inconsistent in the newborns. The cricoid lamina was highest at a location slightly medial to the cricoarytenoid joint in most larynges. In some larynges, it was highest at the JP. The absolute value of AI was less than 0.1 in the majority of the larynges investigated.

The location of the JP showed a unique pattern of asymmetry. Its distance from the anterior midpoint of the cricoid arch (DJP) was greater on the right side than on the left side in the majority of the adult larynges, presenting with positive AI values (Fig 5B). The same tendency was noted for the distance of the JP from the midsagittal plane (WJP) and that from the anterior frontal plane (LJP). This tendency was not marked in the newborns. The absolute value of the AI was less than 0.1 in most cases.

These results indicate that the right cricoarytenoid joint tends to shift slightly downward, backward, and lateralward with increasing age.

Cricothyroid Complex. The angles of the longitudinal axis of the thyroid cartilage against that of the cricoid cartilage (ACTU, ACTL) are shown in Fig 6A,B. At the upper edge, the axis of the thyroid cartilage was shifted to the right posteriorly (positive ACTU values in Fig 6A) in most adult larynges. This tendency was not consistent in newborns. The absolute value of ACTU was 5° or less in the majority of the cases. At the lower edge, there was no directional preponderance in the shift of the thyroid axis against the cricoid axis in any age group (Fig 6B). The absolute value of ACTL varied greatly, and it exceeded 5° in some larynges.

Figure 6C shows the angle of the thyroid cartilage against the cricoid cartilage in the frontal plane (ACTH). Most adult larynges presented positive values, indicating that the thyroid cartilage was tilted to the right against the cricoid cartilage. In newborns, no directional preponderance in tilting of the thyroid cartilage was observed. The absolute value of ACTH varied. It was 5° or greater in some larynges.

There was no directional preponderance in differences between the right and left sides for the distance between the AC and the JP (DJPC) (Fig 6D). The absolute value of the AI for DJPC was less than 0.1 in all cases. The angle of the line between the AC and the JP against the midsagittal plane of the larynx (AJPC) was different between the right and left sides in most larynges (Fig 6E). As described earlier, this line was close to the vocal fold edge. No directional preponderance was noted in the differences of AJPC between the two sides. The absolute value of the right-left difference of AJPC was less than 5° in most larynges.

Figure 7 shows photographs of a larynx with the typical pattern of framework asymmetry for older adults.

DISCUSSION

Asymmetry of the cartilaginous framework of the larynx has been described previously.[1-6] It has, however, not been investigated systematically.

The results of the present study are summarized as follows. The laryngeal framework is asymmetric to a greater or lesser extent in all larynges in all age groups. In newborns, there is no directional preponderance in any pattern of asymmetry.

In older adults, there is a directional preponderance in the pattern of asymmetry. The right thyroid lamina tends to tilt laterally, whereas the left thyroid lamina shows a tendency to tilt medially. As a result, the right tip of the superior thyroid incisure is located anterior to the left tip, the base of the right superior cornu is placed more anterolaterally than that of the left superior cornu, the base of the right inferior cornu is situated more posteromedially than that of the left inferior cornu, and the longitudinal axis of the thyroid cartilage at the upper edge shifts to the right posteriorly against the longitudinal axis of the cricoid cartilage.

The left thyroid lamina is higher than the right at the inferior tuberculum. The cricoid lamina also is higher on the left side than on the right side. The right cricoarytenoid joint is located slightly more laterally, posteriorly, and inferiorly than the left joint. The thyroid cartilage is tilted to the right relative to the cricoid cartilage.

It should be noted that all adults investigated were right-handed. Two questions remain for further studies. Is the pattern of asymmetry observed in the older adults attributed to the right-handedness? If so, how does it occur? The asymmetry of the thyroid cartilage of older men reported by Honjo et al[1] appears to be basically the same as that observed in the present study. They did not report the handedness of their subjects. Since the majority of Japanese people are right-handed, the subjects in their paper, at least most of them, are presumed to be right-handed. A systematic study of the laryngeal framework of left-handed adults is required in order to generalize the relationship between handedness and the direction of asymmetry of the laryngeal framework.

There were some exceptional cases in which asymmetry of the framework was not very marked or the laryngeal framework was deformed to the opposite direction. The reasons for this await further investigation.

It should be noted that the line between the AC and the JP of the cricoid cartilage, which was close to the location of the vocal fold edge, did not show directional preponderance in asymmetry. Furthermore, the degree of asymmetry was not very great. This indicates that there must be some compensatory mechanisms that keep the edges of the bilateral vocal folds relatively symmetric. Further studies of the dynamics in excised larynges and living subjects are required to elucidate the compensatory mechanisms.

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SECTIONAL ANATOMY OF THE LARYNX: IMPLICATIONS FOR THE TRANSCUTANEOUS APPROACH TO ENDOLARYNGEAL STRUCTURES

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A transcutaneous, transcricothyroid membrane approach to endolaryngeal structures is used for several techniques such as placement of electrodes for laryngeal electromyography and transcutaneous Teflon injection of the paralyzed vocal fold. The purpose of this study is to examine the sectional anatomy of the larynx with respect to the cricothyroid membrane and describe a suitable transcutaneous approach to endolaryngeal structures. Sixteen whole organ cadaveric larynges were frozen in clear gelatin to facilitate sectioning. Specimens were sectioned in the transaxial, sagittal, and coronal planes as well as 30°, 45°, and 60° from the transaxial plane. For clinical orientation, the transaxial plane was defined as 90° from the most anterior plane of the cricothyroid membrane. Sections were examined and angles and depths of penetration were measured with respect to the transcutaneous approach to endolaryngeal structures.

KEY WORDS — laryngeal electromyography, larynx, sectional anatomy, Teflon injection.

INTRODUCTION

Several techniques have been proposed that use a transcutaneous approach to endolaryngeal muscles. They include the placement of electromyography (EMG) recording electrodes,[1-3] the therapeutic injection of botulinum toxin for adductor spastic dysphonia,[4-6] and the injection of Teflon into the paralyzed vocal fold.[7] Individual approaches have varied but generally rely upon the operating experience of a laryngologist for proper placement rather than the use of carefully described measurements and angulations.

Kotby[8] attempted to standardize the technique of transcutaneous laryngeal EMG by observing needle placement with direct laryngoscopy in cadaver specimens and recording angles and depths of penetration from the cervical skin. Others have confirmed needle placement by observing the pattern of EMG activity recorded by the needle.[1-3]

The purpose of this report is to describe the sectional anatomy of the larynx with respect to the cricothyroid membrane as it applies to the transcutaneous approach to endolaryngeal structures. The cricothyroid membrane was chosen as a clinical landmark as it is readily identified in most individuals and its relationship to endolaryngeal structures is less variable than that of the cervical skin.

METHODS

Sixteen fresh neck specimens with an intact larynx, hyoid bone, esophagus, thyroid gland, and strap muscles were taken at necropsy. The strap muscles and the thyroid gland were removed and the posterior pharyngeal wall was trimmed. For the purpose of clinical orientation the central axis of the larynx was defined as parallel to a line connecting the most anterior projections of the inferior rim of the thyroid cartilage and the anterior cricoid arch. The transaxial and sagittal planes were at 90° with respect to this axis (Fig 1). The larynx was suspended in a viscous gelatin solution and oriented with the central axis of the larynx parallel to the bottom of the container. The specimens were frozen at −18°C for 24 hours in this position.

The frozen specimens were cut with a sharp band saw into approximately 2-mm sections in the transaxial, sagittal, and coronal planes and at 30°, 45°, and 60° (in an anteroposterior direction) from the transaxial plane. While still frozen, the superior

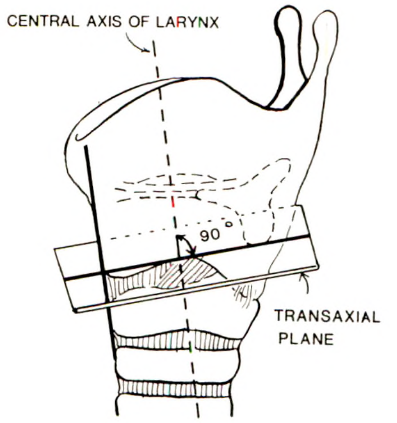

Fig 1. Laryngeal framework with orientation view of central axis of larynx (see text for definition). Transaxial plane is at 90° from this axis.

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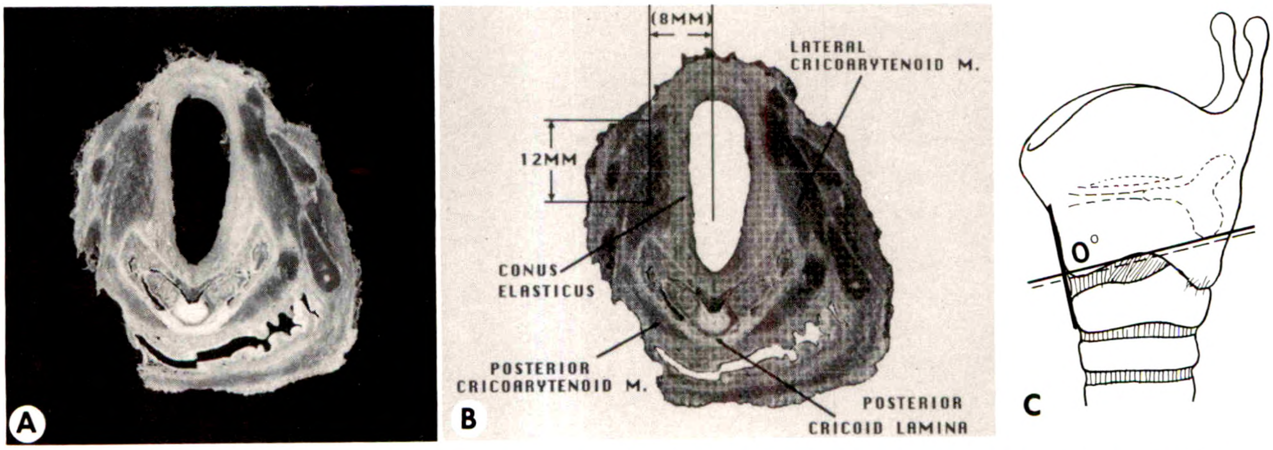

Fig 2. A) Laryngeal section (male), B) labeled image, and C) orientation view through cricothyroid membrane in transaxial plane. Lateral and posterior cricoarytenoid muscles are identified.

surface of each specimen was photographed at a standard distance to facilitate measurements.

The sections through the cricothyroid membrane were chosen from each specimen for measurement. The midbellies of the thyroarytenoid, lateral cricoarytenoid, interarytenoid, and posterior cricoarytenoid muscles were identified. The distance of the midbelly of each muscle from the cricothyroid membrane in a sagittal plane as well as the distance of that sagittal plane from the midline were measured.

Needles were placed in another set of whole organ specimens to the specified depths. The larynges were cut in the midsagittal plane and the position of the needles was confirmed by gross dissection.

RESULTS

The sections are ordered according to ascending angulation from the transaxial plane in sets of three (Figs 2-5). Each Figure includes a photograph of the actual section, a labeled digitalized copy of the section, and an orientation drawing. The pertinent measurements are shown in the Table.

Thyroarytenoid Muscle. In the male larynx fibers of the thyroarytenoid muscle are identified in sections taken at 45° (Fig 4) and 60° from the transaxial plane (not shown). In the female larynx thyroarytenoid muscle fibers are identified in the 30° (Fig 3) and 45° planes. The depth to the midbelly of this muscle is greater in the male larynx (13 mm male, 7 mm female), particularly at the 45° angle (see Table). The sagittal plane of the midbelly of this muscle was 5 mm lateral to the midline in both sexes (Fig 4B). Direct measurements of depth and angle for approach to the thyroarytenoid muscle were taken from the sagittal sections (Fig 5C). In the male larynx the midbelly of this muscle was located 50° from the transaxial plane at a depth of 10 mm, while in the female larynx it was at 40° and 8 mm depth.

Lateral Cricoarytenoid Muscle. The midbelly of the lateral cricoarytenoid muscle is at a shallower angle in the female larynx than in the male larynx. In the male larynx fibers of this muscle are seen in the 30° section and some fibers in the 0° section (Fig 2B), while in the female larynx a larger portion of this muscle is identified in the 0° section. The depth

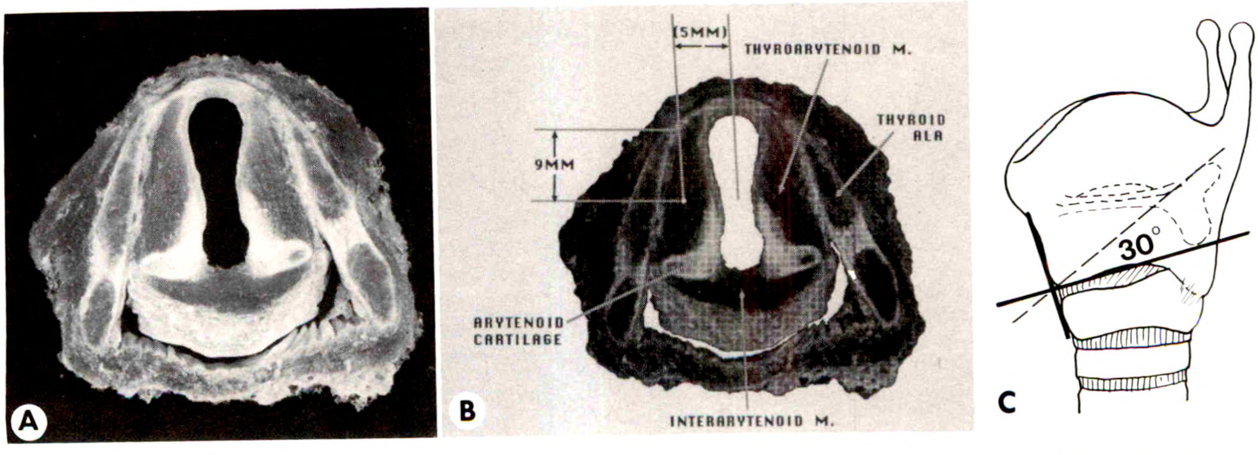

Fig 3. A) Laryngeal section (female), B) labeled image, and C) orientation view through cricothyroid membrane at 30° (anteroposterior) from transaxial plane. Thyroarytenoid and interarytenoid muscles are identified.

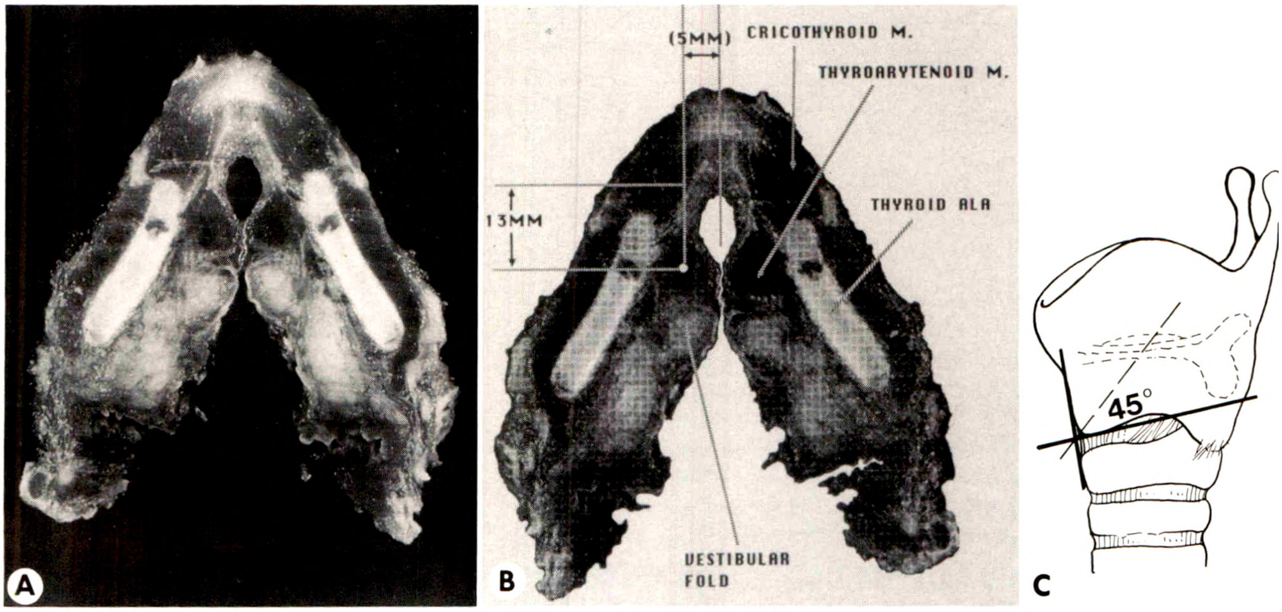

Fig 4. A) Laryngeal section (male), B) labeled image, and C) orientation view through cricothyroid membrane at 45° (anteroposterior) from transaxial plane. Thyroarytenoid and cricothyroid muscles are identified.

of penetration to the midbelly of this muscle was the same in both sexes (12 mm; see Table). The sagittal plane of the lateral cricoarytenoid muscle was slightly more lateral to the midline than that for the thyroarytenoid muscle: 6 mm in the female and 7 to 8 mm in the male (see Table).

Interarytenoid Muscle. The interarytenoid muscle was identified in the 30° plane in both sexes (Fig 3). The depth was slightly greater in the male (29 mm) than in the female (26 mm). The transcricothyroid membrane approach to this midsagittal muscle must cross the mucosa twice, while each of the other endolaryngeal muscles may be reached submucosally.

Posterior Cricoarytenoid Muscle. Fibers of the posterior cricoarytenoid muscle were identified in the 0° plane in both sexes (Fig 2B). Most of the muscle is shielded anteriorly by the posterior cricoid lamina. Only the most lateral fibers of this muscle may be reached transcutaneously.

DISCUSSION

Endolaryngeal muscles reliably can be reached transcutaneously through the cricothyroid membrane with a needle for injection or electrical recording. This approach has been used in the past for the placement of EMG electrodes[1-3,8] and for the injection of botulinum toxin for spastic dysphonia[4-6]

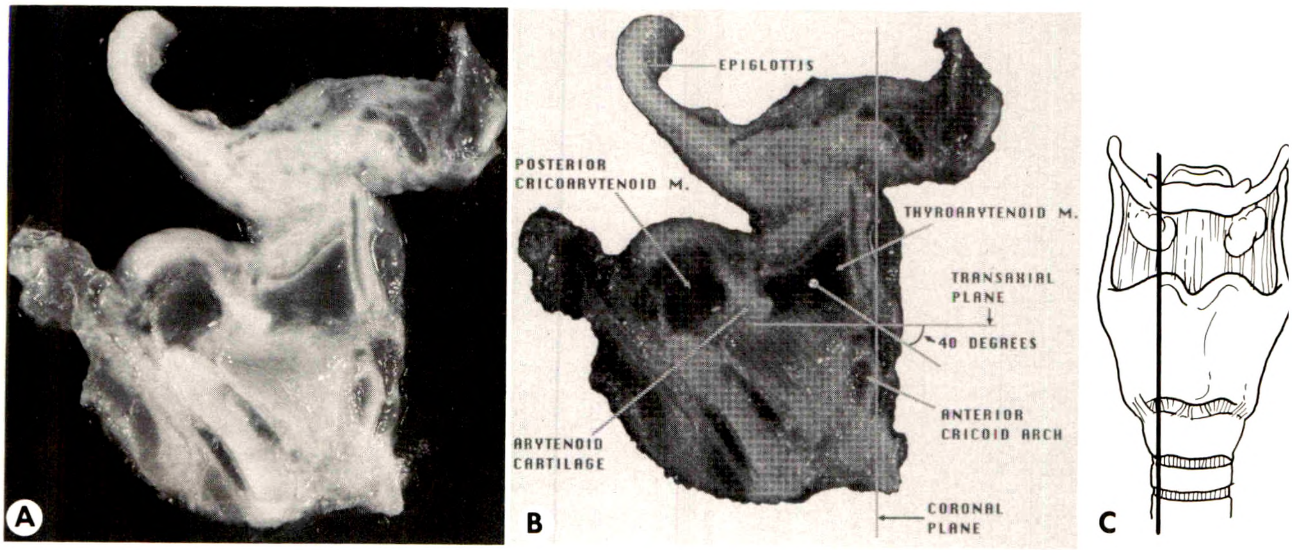

Fig 5. A) Sagittal laryngeal section (female) and B) labeled image approximately 5 mm lateral to midline. Midbelly of thyroarytenoid muscle is identified (circle). C) Angle and depth of approach.

MEASUREMENTS FROM MIDBELLY OF SPECIFIED MUSCLES

	To Cricothyroid Membrane				*To Midline*			
Angle From Transaxial Plane	*TA (mm)*	*LCA (mm)*	*IA (mm)*	*PCA (mm)*	*TA (mm)*	*LCA (mm)*	*IA (mm)*	*PCA (mm)*
Male								
0°	—	12	—	22	—	8	—	8
30°	—	12	29	—	—	7	0	—
45°	13	—	—	—	5	—	—	—
60°	9	—	—	—	5	—	—	—
Female								
0°	—	12	—	20	—	6	—	9
30°	9	—	26	—	5	—	0	—
45°	7	—	24	—	4	—	0	—

TA — thyroarytenoid muscle, LCA — lateral cricoarytenoid muscle, IA — interarytenoid muscle, PCA — posterior cricoarytenoid muscle.

and Teflon for vocal fold paralysis.[7] Clarification of the quantitative anatomic relationships with respect to the cricothyroid membrane is useful to those interested in applying these techniques.

Kotby[8] reported depth and angle of approach to laryngeal muscles from the cutaneous surface of the neck. He assessed needle placement by exposing the interior of the larynx with a rigid laryngoscope and observing the relationship of the needle to certain known anatomic landmarks. We chose the cricothyroid membrane as a reference point because of its more constant relationship to these muscles. Furthermore, using the cricothyroid membrane clinically to define the central axis of the larynx reduces the normal variability in the vertical orientation of this structure. The use of anatomic sections is more precise in identifying endolaryngeal muscle than methods used previously. Despite the differences in methods, our results are similar to those reported by Kotby.

This study shows that the thyroarytenoid and lateral cricoarytenoid muscles may be reached submucosally through the cricothyroid membrane. Use of the anteriormost plane of the cricothyroid membrane for orientation is helpful clinically as it is easily palpable in most individuals. This landmark reduces the variation of laryngeal position and depth encountered with respect to the overlying skin.

The thyroarytenoid muscle can be approached in a sagittal plane approximately 5 mm from the midline at an approximate angle of 50° and a depth of 9 to 13 mm in the male. In the female larynx this angle is shallower (approximately 40°) and the depth is less (7 to 9 mm). The lateral cricoarytenoid muscle can be approached more laterally at an angle more closely approximating the transaxial plane but at a greater depth.

The transcutaneous placement of Teflon for vocal cord augmentation requires the bolus be placed in or just lateral to the thyroarytenoid muscle; therefore, these measurements also provide guidelines for this technique.

The interarytenoid muscle can be reached through the mucosa in the midline at an angle of 30° (Fig 3) and a depth of 26 mm in the female and 29 mm in the male.

Some investigators approach the posterior cricoarytenoid muscle transcutaneously,[3] reaching only the most lateral fibers. The midbelly of this muscle is consistently guarded by the posterior lamina of the cricoid cartilage (Fig 1) and, more cephalad, by the arytenoid cartilage. According to these observations a transoral approach to this posteriorly positioned muscle may be more reliable.

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CURRENT AND FUTURE HORIZONS IN LARYNGEAL AND VOICE RESEARCH

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Voice dysfunction is associated with neuromuscular impairment of laryngeal control and is often difficult to diagnose by indirect examination of the larynx. The newer techniques, currently used in the UCLA–Veterans Administration Medical Center Laboratories, provide some methods that aid in the diagnosis of voice impairment by providing documented objective data. Illustrative case reports of disorders of neuromuscular dysfunction of the larynx are presented and future research needs and direction are discussed.

KEY WORDS — electroglottography, laryngeal research, photoglottography, voice research.

The majority of voice disorders in all age groups are associated with pathologic abnormalities of the laryngeal structures. The diagnosis is often evident upon completion of a history and careful physical examination that demonstrates the anatomic abnormalities. Much more difficult is the diagnosis of voice impairment when there is no grossly evident anatomic abnormality.

In the absence of an obvious cause of a voice disorder, the history becomes even more important, for the patient can be observed and the voice heard simultaneously with the acquisition of data. While in this article emphasis is placed upon objective measurements, there remains no finer computer than that which rests upon our shoulders, when properly programmed and used. In many ways (voice quality, changes in pitch, breathlessness), the patients provide the diagnosis. Of importance also are the nonverbal clues: the patient's appearance, emotional state, and psychosocial, interpersonal, and occupational factors. The diagnosis of neuromuscular abnormalities affecting the larynx may be particularly difficult when only brief glimpses are obtained with the mirror or telescope.

In recent years, major advances have been made in our ability to document the appearance of the larynx. Motion pictures and, more recently, videotape photography of telescopic and fiberoptic examinations of the laryngeal and hypopharyngeal structures allow repeated and stop frame analyses of the dynamic events, with magnification and detail that increase diagnostic precision.[1-5] Commercially available systems provide immediate reproductions that can be shown to the patient and sent to the referring physician. Stroboscopy of the larynx has been used, discarded, and reused in many laboratories. It is now undergoing a resurgence, since new light source and stroboscopic units allow black and white or color videotaping and documentation of the stroboscopic image.[6,7] Unfortunately, a method of quantification of the stroboscopic image has yet to be worked out, but it does assist in differentiating between functional or structural bowing, and may show the cause of persistent dysphonia after laryngeal surgery by showing stiffening of the mucosal wave. Recent work in our laboratories now allows matching of the stroboscopic images with glottographic waveforms.

Among the objective measures currently in use in most voice laboratories are commercially available instruments that provide automated acoustic analysis of jitter, shimmer, and harmonic-to-noise ratio.[8-10] Aerodynamic measures, such as glottal airflow and subglottal air pressure, provide another perspective on voice production from which inferences on abnormal glottal configurations can be made.[11] Electromyographic recordings of laryngeal muscle activity provide useful information about the physiology of specific muscles during phonation.

Measurements that can be directly related to the pathophysiology of laryngeal behavior are highly desirable. Since phonatory dysfunction usually manifests itself in abnormal oscillatory movements, the measurement and analysis of the vibratory pattern of the vocal folds has the potential to provide detailed information on the pathophysiology of the vocal folds during phonation. Movement of the vocal folds can be observed by ultra-high speed filming (4,000 to 10,000 frames/s). Frame-by-frame analysis can yield measures such as glottal area and excursion of selected points on the visible surface of

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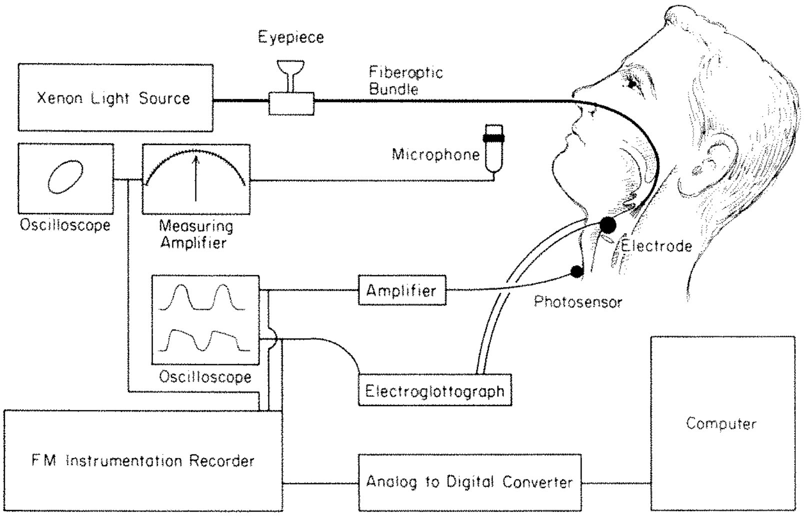

Fig 1. Diagram illustrating simultaneous recording of electroglottography and photoglottography and digitization apparatus.

the vocal folds. Such measures provide valuable information on the pathophysiology of individual patients. Although high speed filming does provide useful data, it is technically difficult to use with untrained subjects or patients, and is especially limited by the considerable time and expense of frame-by-frame data analysis. In fact, the difficulties of this method are so great that more than 40 years after first being used, it is estimated that not more than 5 minutes of ultra-high speed film of vocal fold vibration have been analyzed in detail.

The significant recent advance is the ability to assess the characteristics of phonatory function objectively at the level both of vocal fold vibrations and of the resulting acoustic signal. Two objective measures are photoglottography (PGG) and electroglottography (EGG).[12] The technology of digital recording and computer-assisted signal processing techniques has made feasible the documentation and analysis of such high speed events as vocal fold vibration and acoustic voice signals.[13] Glottographic techniques offer a rapid, relatively inexpensive alternative to high speed photography. Analysis of glottal waveforms, ie, waveforms that describe glottal movement during phonation, provide the potential for measurement of vocal fold activity with little discomfort to the patient. The application of voice and glottographic signal analysis in the evaluation of patient populations is providing data for objective assessment of voice disorders.[14]

The establishment of a voice disorders laboratory requires a broad-based multidisciplinary approach to phonatory physiology and pathophysiology. A well-equipped laboratory provides the investigators with the capacity to document fiberoptic, telescopic, and stroboscopic laryngeal examination, high quality reproductions of acoustic voice signals, glottal airflow and pressure measurements, and glottographic assessment of vibration.[15] Computing facility capability allows simultaneous digital recording of multiple signals for subsequent automated or interactive analysis of data. A diagram of the recording and digitization apparatus is illustrated in Fig 1.

The vibratory movements of the vocal folds during voice production occur faster than the eye can follow, and objective measurements of these vibratory movements offer advantages over purely visual examination techniques.[15] New data are accumulating to confirm the objective measurements of vocal fold vibratory function with EGG[16] and PGG.[17] Electroglottography provides measurements of the changes in electrical impedance across the larynx during vocal fold opening and closing. The dynamic impedance between the two skin electrodes that changes as the vocal folds spread apart and then approximate each other is recorded by EGG. A generally accepted interpretation of the events seen in a normal EGG pattern is seen in Fig 2A.

Comparison of stroboscopic images with EGG confirms the view that EGG signals reflect the variations in vocal fold contact.[18] Photoglottography measures the light transmission between the vocal cords (the glottic chink) during phonation. A light source is placed in the oral cavity, while a photosensor placed on the neck at a level below the vocal cords measures the amount of light transmitted through the cords during phonation. Photoglottography measures the light transmission between the vocal cords (the glottic chink) during phonation. An interpretation of events seen in a normal PGG is demonstrated in Fig 2B.

Comparisons of high speed photography with that of the intensity of light transmitted during PGG is a good approximation of cross-sectional area of the glottis during phonation.[19-21] Photoglottography and EGG are complementary measures; one describes the glottic opening while the other provides information on the glottic closure.[21] The EGG and PGG waveforms also can be used to calculate two other possibly useful measurements: the speed

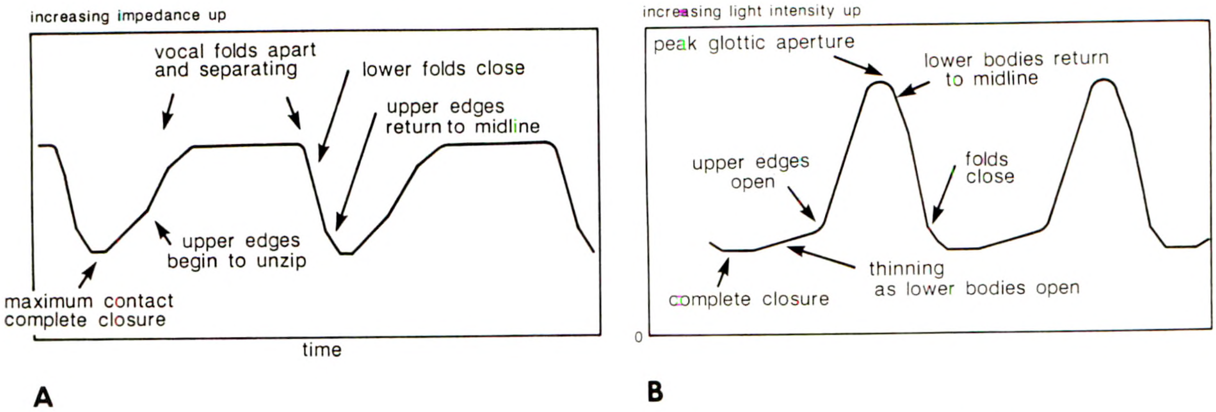

Fig 2. Events identified in normal glottography signals. A) Electroglottography (impedance/time). Idealized waveform modified from Rothenberg. (Rothenberg M. Some relations between glottal air flow and vocal fold contact area. In: Ludlow C, Har M, eds. Proceedings of the Conference on the Assessment of Vocal Pathology, Report 11. Rockville, Md: American Speech and Hearing Association, 1981:88-95.) B) Photoglottography (glottic area curve/time).

quotient and the open quotient. The open quotient represents the fraction of time the vocal folds are open during the glottic cycle, and the speed quotient the time they are opening divided by the time they are closing.

METHOD

Our method for examining a patient with phonatory disorders has been published previously.[12] We have converted from 16-mm motion pictures to $^{3}/_{4}$-inch videotape obtained with a miniature videocamera. The control data from ten normal male volunteers in good physical condition and screened for absence of voice abnormalities has been presented previously.[22,23] All subjects were recorded in a double-walled, sound treated (Industrial Acoustics Corp) room. The subjects vocalized a sustained /i/ while videolaryngoscopic documentation of the gross laryngeal movement and anatomy and PGG and EGG signals were recorded. During phonation of this vowel, the epiglottis is in a forward position, thus minimizing obstruction of supraglottic light for PGG.

The PGG measurement system consists of a Centronic, single element photovoltaic detector with an active area of 50 mm^2, followed by a preamplifier with a bandwidth of around 5 kHz. The preamplifier and photodetector are encapsulated in plastic and electromagnetically shielded. The photodetector is placed on the skin of the neck overlying the cricothyroid membrane. The larynx is illuminated by projection of light through an Olympus flexible fiberoptic telescope inserted through a nostril and positioned so that the glottis fills 50% of the viewing field; a 350-W xenon light serves as the illumination source.

The EGG signals were recorded using a Synchrovoice research electroglottograph attached to the subject by an elastic collar. The two surface electrodes were located on either side of the thyroid alae, and a ground electrode was placed on the side of the neck. At comfortable pitch and loudness levels, the PGG and EGG signals are recorded simultaneously during steady state phonation of the vowel /i/. Both control and patient subjects attempted to match frequency and intensity targets. The glottographic signals were recorded for later analysis on a four-channel FM recorder (Tandberg).

The recorded signals were low-pass filtered at 3 kHz and synchronously digitized at a sample rate of 20,000 points per second. The multipurpose signal analysis program is used to mark several points for each glottic cycle. Gerratt el at[22] described the point of opening of the glottis to airflow. The point of peak opening is determined from the zero crossing of the velocity of the PGG signal. The amplitude of this peak is measured. Baseline signal is determined by the PGG amplitude at the point that the EGG signal indicates minimum impedance (maximum contact across the glottis). The point of closing is marked at the peak deceleration of the PGG signal, which corresponds most closely with the peak closing velocity of the EGG signal.

Fifty cycles of representative phonation are used to acquire the various measurements. The time-related measurements include the period of each cycle, the closed phase, the open phase, the period during the open phase in which the glottic area is increasing, and the period during which the open phase of the glottis is decreasing.

High quality acoustic recordings are made during sustained vowel production in each subject. Vocal tract resistance is calculated from airflow and from estimated subglottal pressure signals. Acoustic, perceptual flow, and pressure data are in the process of analysis for future reports. An example of the normal, simultaneously recorded waveforms of EGG and PGG is presented in Fig 3A. These normal waveforms can be compared visually with those recorded from patients with various central and peripheral pareses, paralyses, and central de-

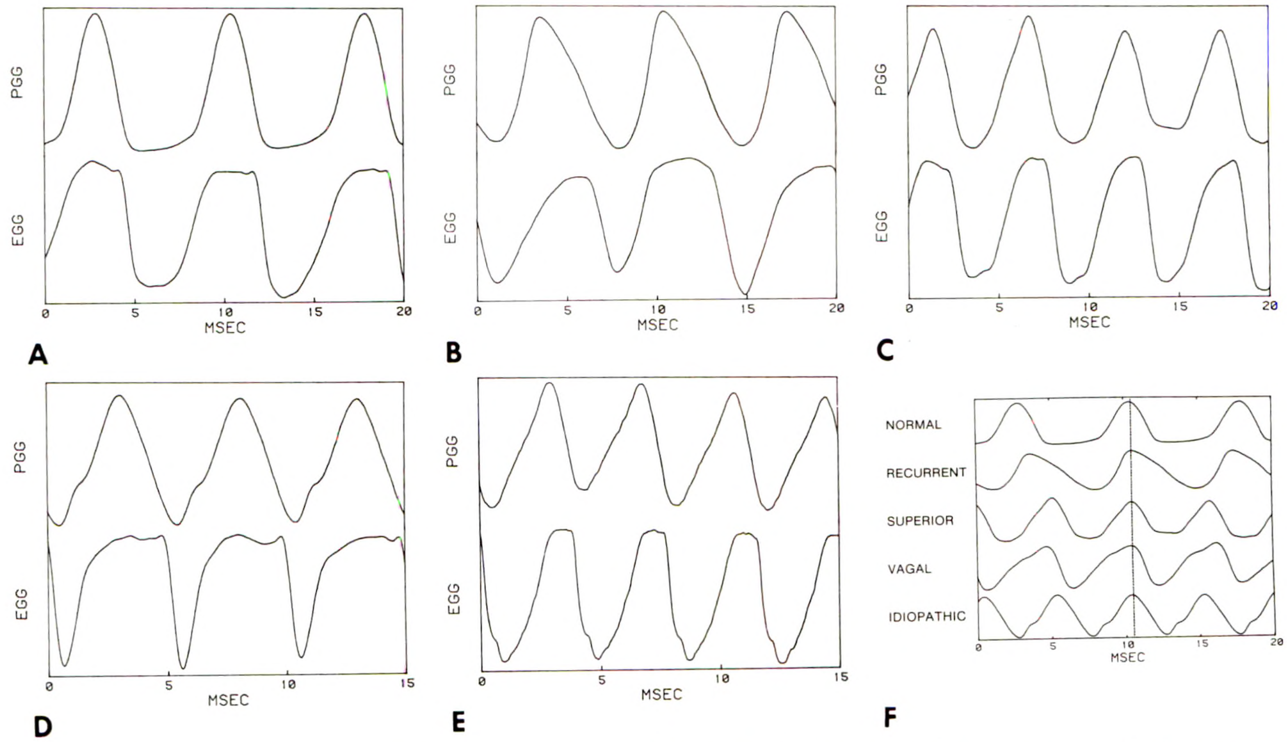

Fig 3. Typical simultaneously recorded electroglottography (EGG) and photoglottography (PGG). A) Normal. B) Recurrent laryngeal nerve section. C) Superior laryngeal nerve paralysis. D) Idiopathic paralysis. E) High vagal nerve section. F) Composite for comparison of PGG patterns.

generative processes (eg, Parkinson's disease, essential tremor).

The EEG and PGG signals recorded in a patient with the recurrent laryngeal nerve sectioned show an open period for most of the glottic cycle (Fig 3B). The duration of the opening phase is less than half the duration of the closing phase. The EGG signal reaches peak impedance at approximately the same time that the projected glottal area is maximum. This differs from the normal pattern (Fig 3A), in which the plateau of the EGG impedance occurs prior to the peak glottal aperture. The EGG signal waveform in patients with recurrent nerve section is less flat than is the normal pattern and occurs early in the duty cycle compared to normal signals.

In isolated superior laryngeal nerve paralysis, there are visibly determinable differences from recurrent nerve paralysis. Figure 3C shows that the baseline glottic closure varies from cycle to cycle. The duration of the opening is longer than the closing cycle. Comparing these to normal signals and to recurrent nerve paralysis, there is greater irregularity from cycle to cycle, often in a recurring pattern over several cycles. Patients with superior laryngeal nerve paralysis have EGG signals that show peak incidence (reflecting maximum separation of the vocal folds) occurring relatively late in the cycle. The plateau of the EGG comprises less duration of the cycle than in the normal subjects and patients with recurrent laryngeal nerve paralysis.

The PGG and EGG pattern of patients with idiopathic paralysis is shown in Fig 3D. These can be compared and contrasted with those seen in patients with recurrent laryngeal paralysis. The glottis is open for most of the cycle. Patients with idiopathic paralysis have prolongation of the opening phase in comparison to the closing phase when compared with the pattern seen in recurrent laryngeal nerve paralysis. There are also changes in the velocity of the opening slope of the PGG. The EGG signals from the idiopathic paralysis patients show that maximal impedance, ie, least contact of the folds, occurred in different patients at variable relationships with the peak of PGG.

The PGG and EGG signals from a patient with high vagal nerve section are seen in Fig 3E. There is no closed period in the glottic cycle, as noted in the variation in the baseline. The duration of opening seen in the PGG is prolonged in relation to closing, with a brief change in the velocity in the opening slope. Lack of vocal fold approximation is reflected in the EGG by a low signal-to-noise ratio.

It is obvious from examination of the signals recorded in patients with carefully selected laryngeal nerve and idiopathic paralyses that specific variations in vibratory pattern are detectable. The various PGG patterns characteristic of each type of paralysis are shown for comparison in Fig 3F.

CASE REPORTS

Three cases are presented to illustrate currently available techniques of objective documentation

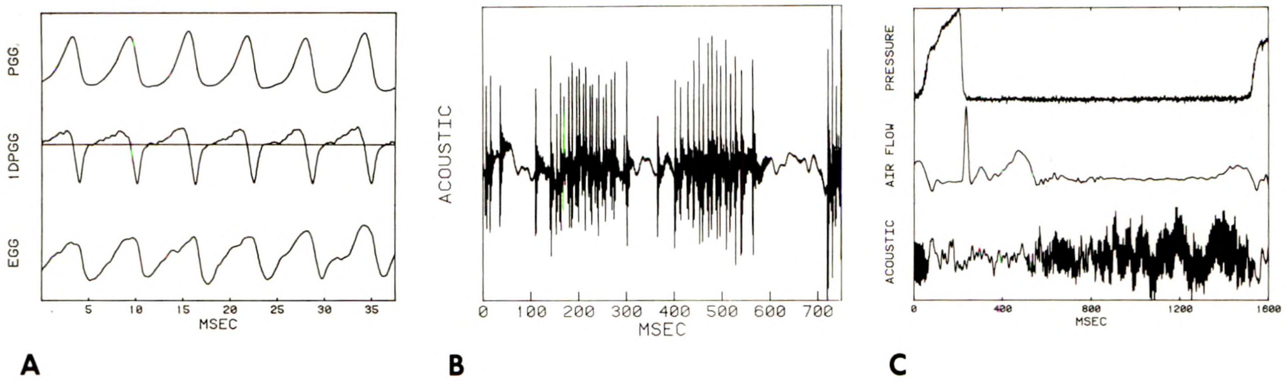

Fig 4. Recordings from patients in case reports. A) (Case 1) Glottographic patterns showing findings typical of Parkinson's disease. PGG — photoglottography, 1DPGG — derived PGG, EGG — electroglottography. B) (Case 2) Acoustic signal recording in patient with myoclonic tremors. C) (Case 3) Pressure, airflow, and acoustic signal recordings in patient with essential tremor.

that are applied to evaluate difficult voice problems and assist in diagnosis.

Case 1. A 67-year-old man who was a heavy voice user presented with a complaint of progressive fatigue and hoarseness. His voice was weak and became more breathy as the day progressed. He had seen several other otolaryngologists concerning his voice abnormality. On minor examination, he had been told that his larynx was normal and he had been referred for speech therapy, which provided minimal beneficial effect. Videolaryngoscopy showed a normal range of laryngeal movement. On close examination during phonation, it was apparent that the vocal processes approximated firmly, but the vocal folds appeared rigid and bowed.

Simultaneously recorded PGG and EGG signals in this patient are seen in Fig 4A. The PGG signal, reflecting the cross-sectional glottal area, indicates that there is an ill-defined baseline during the closed period. There is also asymmetry in the open period, which is better reflected in the opening and closing velocity seen in the derived tracing (1DPGG). The EGG signal, reflecting the vocal fold contact area, is noisy (often with poor vocal fold approximation during the closed phase) and shows maximal impedance (associated with maximum separation of the vocal folds) occurring rather late in relation to the peak glottic area seen in the PGG. These patterns of glottographic signals are typical of those seen in patients who have Parkinson's disease involving the laryngeal muscles.[23,24]

Comment. On the basis of this evaluation, it was recommended that the patient have a neurologic consultation. The patient was told that his voice dysfunction was the result of a neuromuscular control problem. Voice rehabilitation oriented toward the observed disorder was much more effective in helping the patient to cope with his laryngeal dysfunction than had been prior therapy. Two years later, on the basis of subsequent development of other symptoms and signs, the diagnosis of Parkinson's disease was confirmed.

Case 2. A 64-year-old woman was referred for evaluation of spastic dysphonia. The patient, a trained professional singer, had noted gradual onset of extreme difficulty in intensity and pitch. Examination with mirror and the 90° telescope was difficult, but showed hyperadduction of the vocal folds in phonation. The experienced examiner was not successful in observing respiratory movements clearly. A flexible fiberoptic telescope therefore was introduced. Monitoring the patient's hypopharynx showed a regular rhythmic jerking of the larynx at about 2 Hz.

Figure 4B demonstrates the patient's voice signal for a period of 2 seconds during attempted prolonged phonation of the vowel /a/. The jerking movement, also present during quiet respiration, was asymmetrical, primarily to the left side. The magnitude of the myoclonic movement appeared to change with the respiratory cycle, being more prominent at the end of inspiration. During phonation, the attempt to superimpose voicing on the myoclonic movements of the larynx resulted in the observed voice disorder.

Comment. Myoclonus of muscles supplied by the vagal nerve is thought to result from a supranuclear brain stem lesion. Subsequent neurologic evaluation, including a good quality magnetic resonance imaging scan, failed to localize a specific lesion in this patient. In our experience, a specific lesion usually is not identified in patients who demonstrate palatolaryngopharyngeal myoclonus.

Case 3. An 80-year-old woman complained of progressive, gradual deterioration of her voice over the past 7 to 8 years. Her voice was weak and crackling, but at times it sounded spastic in nature and she would have to use force to produce words. The voice problem was intermittent, with good and bad days. At times, she felt that the air "just shut off." She had no previous voice therapy. Objective measurements are demonstrated in pressure, airflow, and acoustic signals recorded in Fig 4C, in which the patient is making the utterance /pa/.

Because of the essential tremor, there is an abnormally long, 400-ms latency present between the onset of the acoustic sound, manifested by the escape of airflow before the normal /a/ is heard.

Comment. Additional objective measurement in this case helped us to understand the dynamic interactions between the tremor and incoordination with breathing.

DISCUSSION

Presently available techniques for documentation and analysis of vocal fold vibrations are providing us with valuable diagnostic information concerning the glottographic patterns of various neuromuscular diseases. The various types of laryngeal paralysis can now be recognized by photographic, video, and glottographic measurements.[23] Characteristic patterns are being used to detect Parkinson's disease even before other neurologic signs and symptoms appear (case 1, Fig 4A). Recognition and separation of the glottographic patterns of essential tremor, myasthenia laryngis, and spastic dysphonia as well as many other neuromuscular disorders of the larynx are now feasible.[15,25]

To better understand the pathophysiologic significance of these observations, we examined an in vivo canine model of vocal fold vibration reported by Berke et al[26] and Moore et al.[7] Lesions of recurrent laryngeal nerve, superior laryngeal nerve, and a combination of the two were produced in the canine model. The PGG and EGG waveform patterns recorded in the canine models were similar to those documented for normal human subjects as well as for human patient data for respective types of paralysis. Simultaneous stroboscopy, triggered from a sharp negative spike in the velocity of the EGG signal, allowed observation of the vibratory movements that were associated with the glottographic waveforms.

In modeled normal phonation, it appears that the velocity of the opening slope of the PGG was determined by movements of the upper edges of the vocal folds. Closing of the glottal aperture reflected movements of the lower bodies of the folds. The increasing impedance of the EGG signal preceded the opening movements of the projected glottic aperture reflected in the PGG signal because the folds opened from below and the lower bodies of the cords reached maximal separation before the upper edges unzipped.

In modeled recurrent nerve paralysis, the flaccid cord did not vibrate in the normal two-mass mode, but rather was pushed laterally as one mass by the subglottal pressure. This was associated with relatively rapid opening of the glottis toward the side of the flaccid fold. The midline of the glottal area shifted toward the flaccid fold. Closure occurred with the upper edge of the normally innervated fold crossing the midline. This resulted in a picture that was very similar to data reported from the few high speed cinelaryngoscopic studies of recurrent nerve paralysis in humans.[27] The pattern of PGG and EGG signals recorded in the model was similar to that seen for the recurrent laryngeal nerve paralysis patients. The glottographic signals recorded from these patients appear to reflect a pathologic vibratory pattern in which the flaccid vocal fold offers little resistance to subglottal pressure. When one fold is paralyzed, the upper edge of the flaccid fold moves laterally relatively early in the cycle, and therefore the opening slope of the PGG reflects primarily the movements of the flaccid cord. The flaccid cord also appears to be delayed in return to the midline, and glottic closure does not occur until the upper edge of the normal vocal fold crosses the midline to approximate the paretic fold. This results in a relatively decreased velocity of the closing slope of the PGG signal. The EGG signal also reflects the rapid separation of the folds early in the sequence of glottal opening.

In the canine model of superior laryngeal nerve paralysis, the upper edges of the folds vibrated 90° out of phase with the normal pattern. There was a clear shift of the posterior commissure toward the side of paralysis with the onset of phonation. On viewing from below, the vocal fold on the side of cricothyroid paralysis first moved laterally. As the subglottic air pulse reached the upper edges of the folds, the midline of the glottic aperture moved markedly toward the side of paralysis. The mucosal wave of both vocal folds then rolled toward the side of intact cricothyroid function. The picture from above showed the epithelial coverings of both folds rolling first to one side, then to the other, with each glottal pulse. The visible glottic aperture viewed from below the folds during stroboscopy traveled from the paretic side to the more tensed side as opening progressed toward the upper edges. As seen from below, it was apparent that the cross-sectional area of the glottic aperture, represented in the PGG signal, resulted from complex interactions of the movements of the lower and upper portions of two folds that, although vibrating at the same frequency, were vibrating out of normal phase with each other. The discontinuity in velocity of the opening slope of the PGG signals and the irregularity of glottographic signal patterns reflect these complex vibratory interactions. Such complex interactions were predicted by Isshiki et al[28] in models of asymmetric vocal fold tension.

With modeled combined paralysis of recurrent laryngeal and superior laryngeal nerves, a combination of the effects of flaccid paralysis with asymmetry of longitudinal tension was observed in the vibratory pattern. The body of the intact, more stretched fold, viewed from below, appeared to vibrate very little, remaining near the midline. As viewed from above, the opening of the upper edges of the vocal folds was distinctly prolonged. Opening progressed from posterior to anterior. Lateral and medial movements of the upper edge of the flaccid fold lagged behind the movements of the more stretched fold. When the lateral excursion of the epithelium of the upper mucosal cover was maximal, the lower body of the intact vocal fold already had returned to midline. Closure of the glottic aperture then occurred very rapidly as the lower body of the intact vocal fold approximated the flaccid fold. The pattern of the PGG waveform seen for vagal nerve paralysis reflected the effects on cross-sectional glottal aperture of prolonged unzipping of the upper edges and phase lag of the lateral and medial movement of the fold on the side of paresis. The PGG and EGG signal patterns and their relationship in the canine model of vagal nerve paralysis approximated the appearance of our vagal nerve paralysis patient's data seen in Fig 3E. This pattern of the EGG signal in relation to the PGG signal appeared to be associated with lack of firm approximation of the bodies of the folds so that the EGG signal reflected primarily the degree of contact of the upper edges. Because of the prolonged opening of the upper fold edges, maximal separation of the epithelial edges occurred relatively late in the cycle, at a time when medial movement of the body of the intact fold already was reducing the cross-sectional glottal aperture. Soon thereafter, contact of the lower bodies of the folds occurred. Thus the plateau of the EGG occurred late in the duty cycle and was of relatively short duration.

The differing patterns seen in the PGG and EGG signals from patients with specific lesions of the laryngeal nerves are consistent with effects predicted by animal model studies. The data from the patient group with idiopathic paralysis are most consistent with the conclusion that these individuals had a lesion that affected motor innervation to both the intrinsic laryngeal muscles and the cricothyroid muscles on the same side of the larynx. The glottographic patterns of vibration were most similar to those seen in patients with vagal nerve section. Previous reports have suggested that the lesion in idiopathic paralysis of the larynx is usually a high vagal or a central lesion.[3]

What can be anticipated for the future in voice research? What should be the future priorities? Already, multiple quantitative measurements determined from glottographic signals are being made, and additional data will be published.[23] These include the open quotient, speed quotient, and shift quotient.

The speed quotient appears to be sensitive to gross variations in neuromuscular tension of the vocal folds in specific neuromuscular disorders. With the shift quotient it is now possible to interlock the PGG, EGG, and videostroboscopy wave motion.[29] The objective quantification consistently shows a close relationship with the glottographic waveforms of the various neuromuscular disorders, thus allowing mathematic analysis. These quantitative measures allow pretherapy and posttherapy measurements, thereby determining changes effected by the treatment.

The future should allow us to quantify both in animal models and in patients the effectiveness or lack thereof of hormonal, pharmacologic, rehabilitative, and surgical therapy. Cooperative team investigative efforts should include an otolaryngologist, an endocrinologist, a neurologist, a physiologist, and a neurophysiologist working with a voice scientist in order to gain a greater understanding of the causes and correction of voice disorders in health and disease.

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CONTRACTILE PROPERTIES OF CANINE THYROARYTENOID MUSCLE REINNERVATED FROM THE ANSA CERVICALIS

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In an attempt to obtain data on the contractile properties of vocal fold muscle reinnervated from the ansa cervicalis, we severed the recurrent nerve and connected its distal stump to the ansa cervicalis by an end-to-end anastomosis in a series of dogs. Each dog was allowed to heal for 5 months. Then the section of the thyroid cartilage on which the vocal fold muscle inserted was detached, connected to an isometric force transducer, and activated by indirect stimulation successively on both the operated and unoperated sides. A series of twitch contractions was recorded from each side. In two dogs no contractile response was obtained. In the three others, the twitch contraction time was increased significantly, by 23% to 60%. In two of three dogs, the operated muscle was significantly weaker than the unoperated muscle. The reinnervated thyroarytenoid muscle changed its speed in the direction of the donor sternothyroid muscle.

KEY WORDS — larynx, muscle, reinnervation, vocal cord.

INTRODUCTION

Studies of laryngeal reinnervation primarily have used electromyography (EMG) and visual examination of vocal fold movement as performance measures to determine the results of experiments. Study of movement is ambiguous in that it may be difficult to specify what muscle has caused the observed movement, since muscles external to the larynx may interact extensively with the activity of intrinsic laryngeal muscles. Furthermore, it is difficult to correlate the results of EMG potentials with the level of mechanical activity of the muscle.[1] While these experiments study contraction that is usually anisometric, EMG signals can be related to the level of muscle activity primarily in isometric contraction,[2,3] and even then the relationship is one of proportionality, so that the absolute level of force produced cannot be inferred from EMG. Our study had two objectives: 1) to evaluate the success of reinnervation through direct recording of the force produced by the muscle in isometric twitch contractions and 2) to obtain more precise information than is available from studies of EMG or vocal fold movement. No previous experimenters have recorded the mechanical contraction properties of laryngeal muscle reinnervated by anastomosis. We selected this procedure because it is impossible to evaluate reports in which EMG activity is observed without corresponding movement of structures. This may result from joint fixation. Similarly, some investigators observed movement, while the results of EMG and histologic observations did not support attribution of the movement to the muscle in question ("false positive" results).[4,5] Direct observation of muscle contraction is the best procedure for verifying whether the operation in question has succeeded in reinnervating the muscle meaningfully.

We collected data to evaluate the effects of reinnervation on the contractile properties of the muscle. The contractile characteristics of muscle are determined in a dual manner, by the character of the motor neurons that innervate the motor unit and by the character of the pattern of activation to which the muscle is subjected.[6,7] Both factors may be altered when a muscle is reinnervated from a donor muscle that has lower motor neurons of different types and perhaps activity patterns of a different type. In a number of experiments extending from the early work of Buller et al,[8] it has been observed that when muscles are reinnervated from motor nerves that previously innervated muscles of a different contractile type, there occurs some degree of adaptation of the contractile properties of the reinnervated muscle. They become more similar to those of the muscle previously innervated by the reinnervating motor nerve.[9] One study has examined the effects of pedicle nerve-muscle grafting on the thyroarytenoid and posterior cricoarytenoid muscles. Grafts from the cricothyroid muscle were inserted into the thyroarytenoid and posterior cricoarytenoid muscles of four dogs. After 10 to 16 months it was found that these muscles exhibited contractile properties appropriate for the cricothyroid muscle,[10] ie, a thyroarytenoid muscle contraction time of 36.3 ms, as opposed to the usual contraction time of about 14 ms.[11,12] For the posterior cricoarytenoid muscle this implied little change, since its contractile properties are normally very similar to those of the cricothyroid muscle in dogs,[13] although its histochemical properties were also changed in the direction of those of the cricothyroid muscle. However, since the contraction time of ca-

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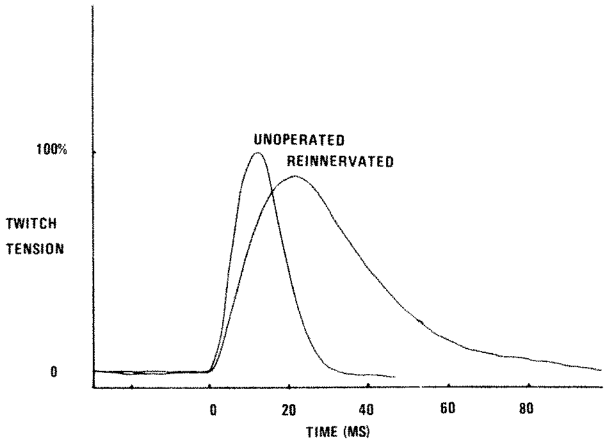

Twitch contractions of unoperated and reinnervated vocal fold muscles of dog 3. Abscissa is marked with zero at beginning of contraction and ordinate is scaled in terms of percent of peak twitch tension of unoperated side. Ordinate zero is level of passive tension in muscles.

nine thyroarytenoid muscle is normally much faster than that of the posterior cricoarytenoid and cricothyroid muscles, it appeared that the reinnervated thyroarytenoid muscle had largely assimilated to the cricothyroid muscle in regard to the contractile properties studied. The histochemical evaluation included only succinic hydrogenase. The superior laryngeal nerve rarely is used in reinnervation,[1] and the results of the use of nerve-muscle pedicles have been mixed.[4,14] In our own study we chose to perform reinnervation from the ansa cervicalis, a nerve that usually supplies motor innervation to strap muscles and has been used with some success in previous studies.[15-17]

METHODS

The right recurrent nerve in five dogs was sectioned and the trunk of the distal section was sutured to the ansa cervicalis in an end-to-end anastomosis. After closing, the wound was allowed to heal. After 5 months, the animals were anesthetized with pentobarbital sodium (30 mg/kg) and an incision was made from just above the level of the hyoid bone to just above the sternum. Additional anesthetic was administered intravenously as necessary. A rectangular incision was made in the thyroid cartilage to permit separation of the section of the thyroid cartilage on which the thyroarytenoid muscle inserted. This rectangle was attached to a 2-0 polyester suture that was led to an isometric force transducer. The arytenoid cartilage was fixed to the cricoid cartilage so as to obtain isometric contraction. The ansa cervicalis and the recurrent nerve were stimulated supramaximally as described in more detail below, to test the contractile properties of the muscle after reinnervation. The signals from the force transducer were digitized by a signal-processing computer (Data 6000) and recorded on diskettes for analysis.

TABLE 1. STRENGTH OF CONTRACTION

Dog	*Reinnervated Tension/Unoperated Tension (%)*	*Significant at .01*
1	48	+
2	103	–
3	82	+

RESULTS

In three dogs (4, 5, 2) no response was observed when the ansa cervicalis was stimulated proximal to the anastomosis. Much scar tissue was observed around the anastomosis in dog 4, and no mechanical response was observed to stimulation on either side of the anastomosis. In dog 5, although the anastomosis appeared healthy, no response in the larynx was observed to stimulation on either side of the anastomosis. Stimulation proximal to the anastomosis did not evoke any contractile response in dog 2, but stimulation distal to the anastomosis did. In this dog the caliber of the nerve in the region of the anastomosis was smaller than on either side. The reason for the disparity is unclear but perhaps is due to conduction block at the constriction.

For dogs 1 and 3 relative values of twitch contraction force and contraction times were evoked by stimulation of the ansa cervicalis, and for dog 2 the corresponding values were evoked by stimulation of the distal stump of the recurrent nerve. Although the absolute values or force may vary somewhat from dog to dog, it is assumed that the left and right values for the same dog will be similar. Thus, these values are reported in relative terms by using the unoperated left thyroarytenoid muscle as the control in each case and reporting the twitch force and contraction time of the operated right thyroarytenoid muscle as a percent of the corresponding value for the left side.

In every case, the twitch contraction time was significantly longer for the muscle that had been reinnervated from the ansa cervicalis. This effect was smaller in the muscle that could not be activated through the anastomosis. The Figure illustrates the difference between unoperated and reinnervated muscles in the time course of muscle twitches obtained from dog 3.

In two of three cases, the operated muscle produced a significantly weaker twitch than did the unoperated muscle. The small difference for dog 2 was not significant (Table 1). This muscle also exhibited a change of contraction time that was relatively smaller than that found in the other muscles, although it was significant (Table 2).

It should be noted that while our measurements of an average contraction time of about 12.5 ms for normal canine vocal fold muscle are close to those of Hast[10,11] and Martensson and Skoglund,[18] who found mean contraction times of 14 ms, they are

TABLE 2. SPEED OF CONTRACTION

Dog	Contraction Time (ms) Unoperated	Contraction Time (ms) Reinnervated	Increase (%)	Significant at .01
1	12.63±0.37	17.81±0.45	41	+
2	12.20±0.54	15.39±1.09	23	+
3	12.77±0.31	20.29±0.63	60	+

about one half of those found by Alipour-Haghighi et al[19] (25.2±5.6 ms) in a recent in vitro study.

Three factors probably account for this difference. First, Alipour-Haghighi et al measured the contraction time, not from the onset of contraction as other investigators have done, but from the onset of stimulation, thus including the latent period as part of the contraction time. Alipour-Haghighi et al state that the time between the stimulation and the onset of contraction amounted to about 5 ms in their data (personal communication). Second, their samples were taken from the medial section of the canine vocal fold, which contains more type 1 fibers than the lateral section,[20] and thus the contraction time would be expected to be slower. Lastly, in the present study, as in those of Hast, we measured twitch contraction times in series of twitches (ten pulses/second) rather than in individual twitches separated by about 5 minutes as in the study of Alipour-Haghighi et al; this might account for a small difference in contraction time.

DISCUSSION

Since both canine vocal fold muscle and sternothyroid muscle are composed predominantly of muscle fiber types that are fatigue-resistant, we did not test muscle fatigability of the reinnervated muscles. Moreover, although observations on cat thyroarytenoid muscle have been published,[21,22] there were no normative values for canine vocal fold muscle to which obtained values could be compared. We have obtained values that have been reported elsewhere.[23] Other research indicates that fatigue resistance of skeletal muscle is not affected by reinnervation.[24] Apparently this property is part of a biochemical stratum of muscle metabolism that is not plastic under the effects of reinnervation, although the effects of reinnervation on contraction speed were clear in the present study.

In these experiments, the distal trunk of the recurrent nerve was anastomosed end-to-end with the branch of the ansa cervicalis that normally innervates the sternothyroid muscle. In retrospect, this muscle may not be the best choice, because the sternothyroid muscle in usual contraction would serve to pull downward on the thyroid lamina; thus it would serve partly as an accessory abductor.[16] However, it is not clear that this movement would produce a failure of the anastomosis rather than simply an inappropriate pattern of activity of the vocal fold muscle. The thyrohyoid branch may be a more appropriate choice, because its contraction raises the thyroid cartilage with the hyoid bone fixed and its EMG activity has been shown to be similar to that of the laryngeal adductors.[16]

The above discussion assumes a separation of the abductor and adductor functions of laryngeal muscles and the recurrent nerve. This, of course, is unobtainable through anastomosis to the recurrent nerve external to the larynx. It is obtainable through connection of the reinnervating nerve to the separate adductor and abductor branches of the recurrent nerve within the larynx, eg, phrenic to the abductor and a branch of the ansa to the adductor branch. Such procedures have been carried out by Rice,[1] Marie,[5] Rice and Burstein,[15] and Crumley[16] with some success.

Physiological observations by Hast indicate that both the canine thyrohyoid and sternothyroid muscles have contraction times of about 50 ms.[25] If the histochemical composition of the strap muscles in dogs is similar to that in humans (the myoglobin content at least is similar),[26] then this observation is consistent with that of Hisa and Malmgren[27] that about two thirds of the fibers in the human sternothyroid muscle were type 1, hence relatively slow-contracting and fatigue-resistant. Reinnervation from nerve fibers that ordinarily innervate the sternothyroid muscle in the present study resulted in a slowing of the contractile characteristics of the vocal fold muscle, although not to a speed as slow as that of the strap muscles. Even after reinnervation through fibers that ordinarily excite a relatively slow muscle, these muscles were still quite fast-moving. Future studies should examine the effect of reinnervation from the thyrohyoid muscle on the contractile properties of the laryngeal adductors. In the present study it is not clear why the change in the speed of the thyroarytenoid muscle was less than that found by Sato and Hisa,[9] when the donor muscle was presumably even slower than the cricothyroid muscle used by them. Several hypotheses are possible. 1) The different nature of the surgical procedure may have affected the nature of reinnervation; for instance, there might result a different type of motor end plate configuration from the nerve-muscle pedicle. 2) The thyroarytenoid muscles in our study might have become slower with further delay before examining them, so that the difference was only the result of the time chosen for harvesting the material. 3) The results may differ because of the different donor muscles chosen in the two studies or the resulting pattern of activation of the muscle, which could be evaluated, eg, by chronic EMG implantation and recording.

A serious disadvantage of our procedure for recording contraction is that it is destructive and successive measurements cannot be made on the same animal to trace the course of reinnervation. It is not necessary for this to be the case. Electromyography, which can be repeated in successive evaluations, does not provide an index of the actual level or time course of contraction. We are considering alternative forms of mechanical measurement that would make possible successive measures of the mechanical response of the muscle so that the time course of recovery after surgery can be evaluated more systematically.

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MODEL OF A NEW GENERATION OF TRACHEOSTOMY AND ENDOTRACHEAL TUBES

A PRELIMINARY STUDY OF SENSORS TO MONITOR OBSTRUCTION

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The feasibility of monitoring cannula obstruction was studied by conversion of the electrical resistance of substances that are capable of causing obstruction into audible auditory signals. Copper-nickel-gold electrodes were thermal pressed onto polyimide-based flexible films placed as 1-mm wide strips along the inner surface of tracheostomy and endotracheal tubes. The responses were fed into a resistance-to-frequency converter (IC 555 timer), and the output was frequency selected to generate auditory responses through a piezoelectric beeper. Quantitative estimates of responses from the IC output were computer averaged. Instantaneous obstruction detection was made possible by the immediate responsiveness of the device in the presence of obstruction.

KEY WORDS — endotracheal tube, monitoring of obstruction, tracheostomy tube.

INTRODUCTION

Postoperative tracheostomy care still is plagued by reports of fatalities due to cannula obstruction.[1-5] A number of causes have been implicated, among which is a combination of inadequate humidification, administration of oxygen, and viscid respiratory secretions contributing to the formation of dry mucous plugs. Adequate humidification is ensured by periodic instillation of normal saline, a mist collar, or a tent; and cannula patency by frequent suctioning. Endotracheal intubation as an alternative is being employed increasingly, especially since the 1970s, for such conditions as acute epiglottitis.[6-9] This is because of the advances made in the development of intensive care facilities,[10-16] including avoidance of surgical risk, reduction in morbidity, and shortened hospital stays.[17] However, this procedure, too, is associated with instances of tubal occlusion.[9,11,16] The twin aims of this report are 1) to recommend a continuous monitoring device that can track cannula lumen patency, and 2) to alert health care personnel to the risk of cannula occlusion.

MATERIALS AND METHODS

An electrode sensor was coupled to an IC 555 timer, the output of which was fed to a high pass filter complex, Cf and Rf, respectively, before being transmitted to a piezoelectric beeper (Fig 1). A small resistance is coupled to R1, in series, to protect the discharge transistor at pin 7 (not indicated in Fig 1). Frequency response is given by the formula $f = 1.44/([R1 + 2R\text{ sensor}]\,C1)$.

An uncuffed tracheostomy tube (10 mm inner diameter, Portex, Hythe, Kent, England) served as a model for the study. After the tube was split open longitudinally, copper-nickel-gold electrodes thermal pressed onto polyimide-based films (Minolta Camera Co, Tokyo) were adhered firmly as 1-mm wide strips to the inner surface of the wall of the

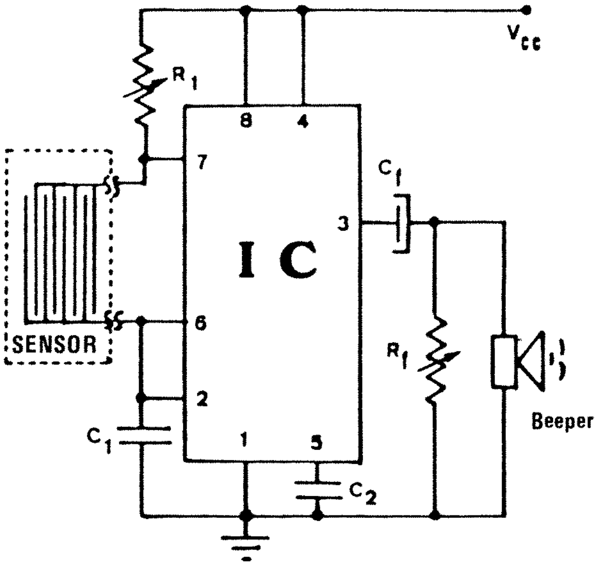

Fig 1. Circuit diagram of device. Cf — filter capacitor, Rf — filter resistor, R1 — resistor, C1 — capacitor, Vcc — power supply. Cf and Rf enhance sensitivity of sensor. Output from R1 and C1 determines frequency of IC 555 timer.

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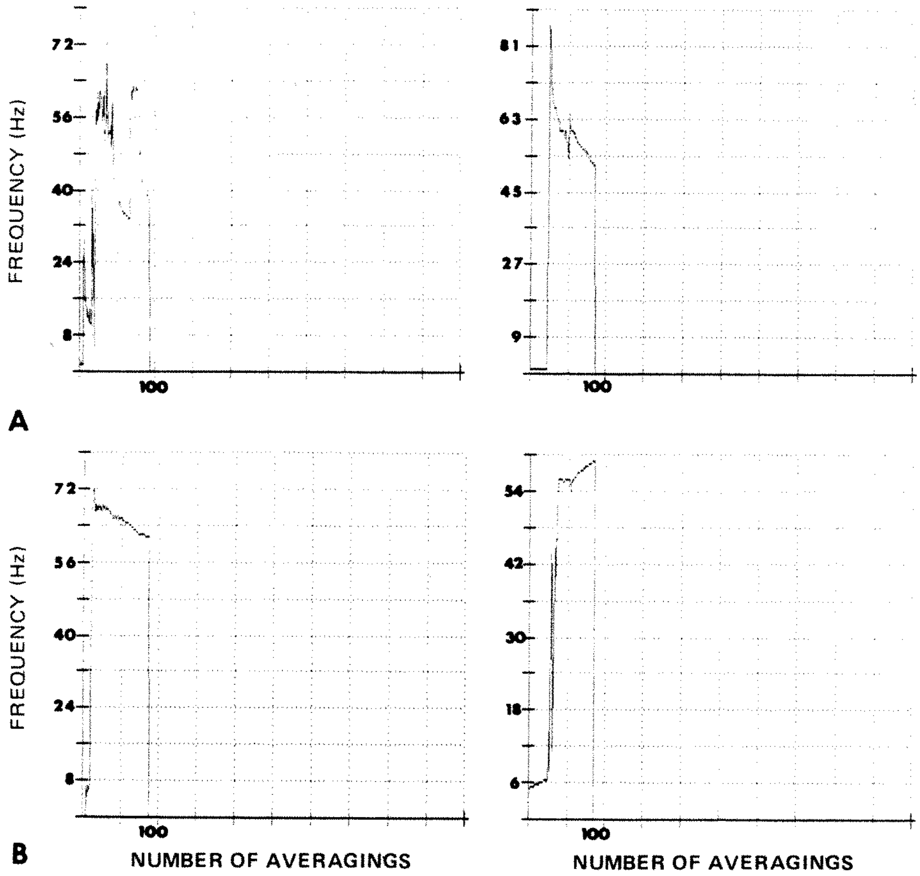

Fig 2. Responses of in vitro study on A) mucus and blood clot and B) tracheal mucosa, granulation tissue, and normal saline.

tube with cyanoacrylate as an adhesive. The electrodes were prepared by thermal pressing copper (35 μm) onto the polyimide film (25 μm), followed by nickel (3 μm), with gold (0.3 μm) forming the surface layer. Four strips were placed along the entire length of the tube from the proximal to the distal end, one in each quadrant of the tube, with the distal ends of the electrodes bent back 90° to line the tip of the distal end of the tube. Finally, the cut ends of the tube were approximated. The electrodes were paired alternately and then connected to a piezoelectric beeper through a frequency converter and a high pass filter. The frequency filter eliminates false alarms. The responses were averaged and analyzed with an IBM PC XT model 286. In vitro individual analysis of each of the following substances was done: 1) freshly collected mucus, 2) clotted blood, 3) tracheal mucosa with granulation tissue, and 4) normal saline, placed within the lumen of the tube. Samples were computer averaged 100 times after a set latency period. In vivo studies were done on healthy adult dogs. After an intramuscular dose of ketamine (15 mg/kg) and an intravenous dose of thiamylal sodium (5 mg/kg), an endotracheal tube was passed and a tracheostomy done. Next, the tube was placed in the trachea. Responsiveness of the tube was studied with the tube fully patent and with normal saline instilled. The tube was left in situ continuously for about 6 hours, and during this time, any alarm caused was analyzed by inspection of the tube for signs of obstruction, which in our study were found to be due to blood clots. The response characteristics were recorded and analyzed as in the in vitro analysis. The responses obtained in in vitro studies are shown in Fig 2 and those in the in vivo studies are depicted in Fig 3.

RESULTS

The various responses, and the patterns obtained using in vitro testing, showed that all the substances tested exhibited a frequency of less than 100 Hz. The in vivo study revealed that the frequency was less than 8 Hz when the tracheal cannula was totally patent, while the responses of normal saline and blood clot were found to correspond to those obtained in the in vitro study. The responsiveness of the tube was found to be instantaneous, in both the in vitro and the in vivo groups. The distinction between cannula patency and lack of it was shown by the fact that the alarm was not activated when the tube was fully patent, while the presence of obstruction resulted in the alarm's being activated, as observed in the in vivo study group.

DISCUSSION

The hazard of obstruction is always present, particularly in patients with long-term tracheostomies,[3] and the majority of the postoperative complications occur in children under the age of 5.[1] A

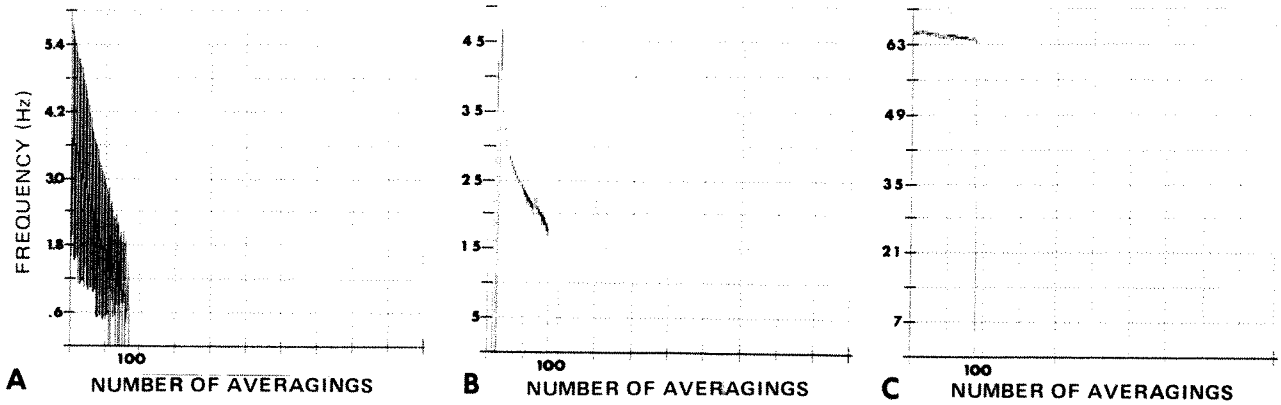

Fig 3. Responses of in vivo study of tube A) fully patent, B) instilled with normal saline, and C) with cannula obstructed by blood clots.

higher incidence of complications is seen in patients under 1 year of age,[3] and occlusion of the cannula presenting as an early as well as a late complication has been recorded.[5] Frequency of obstruction varies from 2.7%[1] to 10.9%.[3] Common causes of occlusion include inspissated mucous plugs or crusts and granulations. Though nasotracheal intubation is being used increasingly in the relief of upper airway obstruction, it also is associated with blockage of the tube by mucus.[9,16] Occlusion can go undetected, even with stringent postoperative care, and since it is of utmost importance to be able to detect obstruction, we suggest a new adjunct to improve postoperative management. This method detects obstruction instantly without any latency. The electrodes are paired alternately, and any obstructing material between any one pair activates the system. The responses are picked up with no loss of latency by the beeper (residing outside the lumen proper), which transforms these changes into audible signals. The polarization effects are minimized, because electrical current flows in opposite directions during charging and discharging of the capacitor during each oscillation cycle. Operating responses can be adjusted by changing R1, and since the frequency increase is of three orders of magnitude, incremental obstruction cannot go undetected. Computer averaging was done to analyze output data and to record differences in responses between individual substances tested. However, in the routine use of such a device, computer averaging is superfluous, because an audible signal is all that is needed to indicate the presence of cannula obstruction. Normal saline was studied since it is used routinely in humidification. Finally, if foreign bodies are suspected in the lower respiratory tract and tracheostomy was performed as an emergency procedure, care should be exercised, because this tube cannot detect every type of foreign body that can be coughed up following a tracheostomy. In such cases, due attention should be given to maintaining a patent cannula until removal of the foreign body, after which this tube can aid in detection of obstruction.

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IMAGING CASE STUDY OF THE MONTH
FACIAL NERVE HEMANGIOMA

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INTRODUCTION

Clinical differentiation between Bell's palsy and unilateral facial nerve palsy due to tumor is sometimes difficult.[1] Slow progression of paralysis, absence of recovery after 6 months, and ipsilateral recurrence all may be important clues of a tumor, but none is specific. Good quality imaging of the temporal bone can be extremely helpful in establishing the diagnosis and minimizing the exploration.[2] It is important to differentiate facial nerve neuromas from vascular tumors, as the timing of surgical therapy may be very different.

CASE REPORT

A 47-year-old woman noted right-sided facial weakness for 8 months, beginning at the corner of her mouth and becoming total over 3 to 4 months. Tearing in the right eye was initially poor but subsequently improved. She had a computed tomogram at another institution that was reported to show normal findings. She did not improve with a trial of corticosteroids.

Her hearing, speech discrimination, and results of auditory brain stem response audiometry were normal. Electroneuronography showed a 97% denervation on the right.

A high-resolution CT showed a 1.3-cm soft tissue mass in the geniculate ganglion region with associated bone changes characteristic of hemangioma or vascular malformation of the region (see Figure, A). An air-CT cisternogram showed no extension of the tumor into the internal acoustic canal (IAC).

A middle fossa exploration of the facial nerve revealed a vascular tumor replacing the geniculate ganglion and the labyrinthine portion of the facial nerve. The tumor was resected, and the nerve defect repaired with a greater auricular nerve graft. Histologic sections showed a vascular malformation/hemangioma.

Her facial movement began to return 7 months postoperatively, and she has normal resting tone and good voluntary movement with mild synkinesis after 1 year.

DISCUSSION

Intratemporal vascular tumors are called hemangiomas when composed of thin-walled vascular spaces, and vascular malformations when composed of thick-walled vascular spaces lined by a single layer of endothelium surrounded by fibroblasts and collagen. An absolute distinction cannot always be made between the two, because they may coexist in a single lesion (see Figure, C). Hemangiomas may be categorized further into cavernous and capillary types by the predominant size of the vascular spaces. Vascular tumors often grow among bone trabeculae and may form bone. In the latter case, they have been called ossifying hemangiomas.[3]

Hemangiomas or vascular malformations along the intratemporal course of the facial nerve once were thought to be rare.[4,5] Recently, they have been seen in some series as often as the much better-known neuromas.[2,3,6]

These tumors occur most often at the geniculate ganglion region, next most often in the IAC, and least often in the tympanic and mastoid segments. They are usually small tumors under 1 cm in diameter. On CT, they may show subtle findings that are often missed. These include irregular and indistinct bone margins, honeycomb bone (see Figure, A) and intratumoral bone spicules.[2]

Other common benign tumors in the geniculate region include the neuromas[7] and the congenital epidermoids,[8] which are usually more obvious lesions with sharper bone margins.

In the IAC, vascular tumors have been well demonstrated as high-signal lesions on magnetic resonance imaging.[2] Although they have been well imaged on high-resolution CT, such tumors have not been demonstrated consistently well on MRI along the fallopian canal. Hence, when imaging for a

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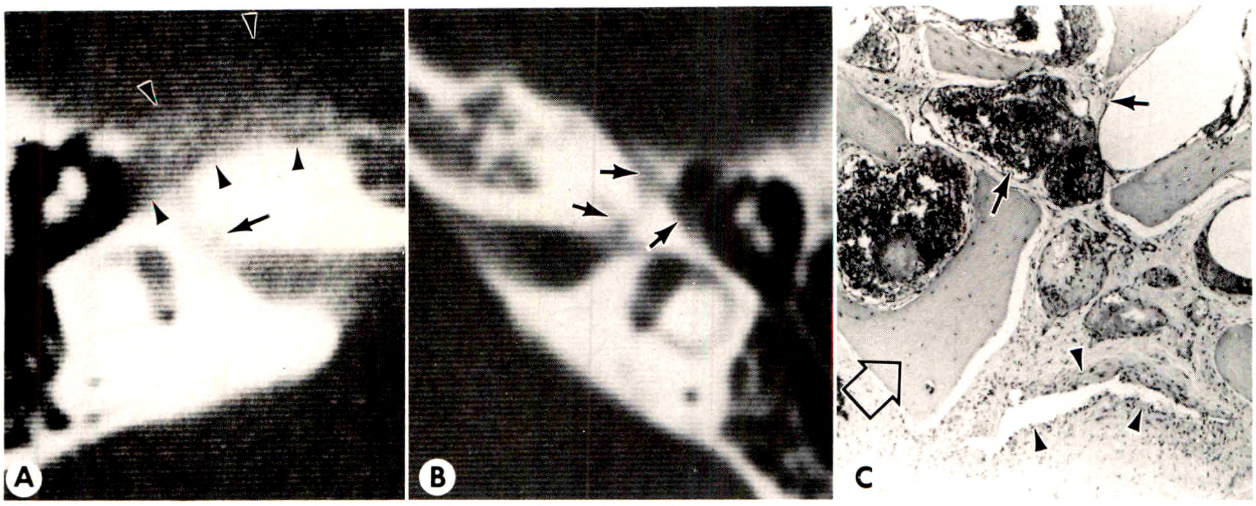

A) High-resolution computed tomogram of right geniculate ganglion region showing 13 x 5 x 4-mm mass with honeycomb bone involvement (arrowheads) and 3-mm irregular, unsharp enlargement of labyrinthine facial nerve canal (arrow). B) High-resolution CT scan of left geniculate ganglion region for comparison. Normal labyrinthine, geniculate, and anterior tympanic segments of facial nerve canal (arrows) are smooth and delicate. C) Photomicrograph of hemangioma/vascular malformation (H & E, original x10). Thin-walled (arrows) and thick-walled (arrowheads) vascular spaces are interspersed among bone trabeculae (open arrow).

facial nerve tumor not clinically localized, one may begin with MRI from the pons to the parotid gland. One should proceed to high-resolution CT with bone detail if MRI does not demonstrate a lesion.

Small vascular tumors tend to produce the same degree of nerve deficit as a larger neuroma.[2,9] Electromyography classically shows denervation and renervation potentials in cases of vascular tumors.[10] While facial neuromas usually require resection and nerve repair, vascular tumors often can be removed with preservation of facial nerve continuity if operated on early. In another recent case of ours, the patient had immediate improvement of facial movement after removal of a small vascular tumor. However, because of the invasive nature of these tumors, facial nerve resection and grafting may be necessary. While it may be prudent to delay operating on facial neuromas in some cases, early resection offers the best chance for good facial recovery in patients with vascular tumors.[11] Radiographic imaging, therefore, plays an important role in planning and timing therapy.

CONCLUSION

Hemangiomas of the facial nerve produce severe neural deficits while of extremely small size. Any facial paralysis that is not typical of Bell's palsy should be evaluated for a lesion at the geniculate ganglion with MRI and/or CT.

ACKNOWLEDGMENTS — We thank Robin Gaylord for manuscript preparation and Ron Campise for photography.

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PATHOLOGY CONSULTATION

LOW-GRADE AND HIGH-GRADE ADENOCARCINOMAS OF THE SALIVARY DUCT SYSTEM

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Adenocarcinomas of salivary glands are encountered less frequently than adenoid cystic or mucoepidermoid carcinomas. They fall into two well-defined clinicopathologic groups: 1) those arising from intercalated ducts and 2) those from the excretory and interlobular ducts. The former, designated terminal duct adenocarcinomas, are low-grade malignancies found preponderantly in the oral cavity, especially the palate. The latter, designated salivary duct carcinomas, are high-grade neoplasms and arise almost exclusively in major salivary glands, especially the parotid gland.

All salivary gland carcinomas arise from precursor cells found along the course of the salivary duct unit that extends from the juxta-acinar intercalated ducts through the excretory ducts.[1,2] In general, those carcinomas arising from the intercalated duct portion are biologically low-grade (eg, acinic cell,

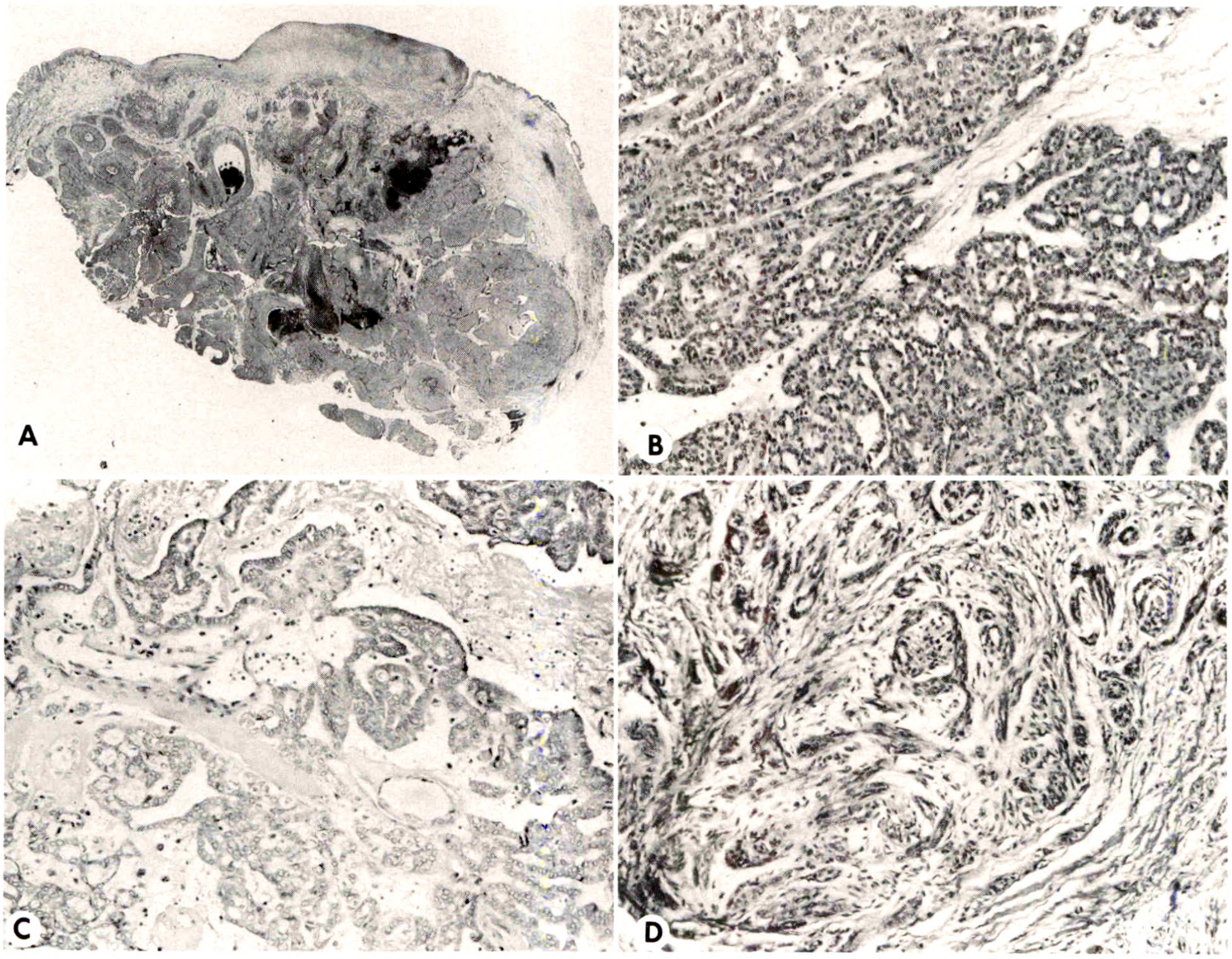

Fig 1. Terminal duct adenocarcinoma A) of soft palate with lobular infiltrative growth, microcystic and solid architecture, and submucosal extension (H & E, original x10); B) showing typical small tubular ducts (H & E, original x60); C) with papillocystic architecture and with cell nuclei showing often-noted vesicular character (H & E, original x60); D) of palate, showing polymorphous character, neoplastic cells that are spindled and less glandular, and small nerve invasion in center of field (H & E, original x60).

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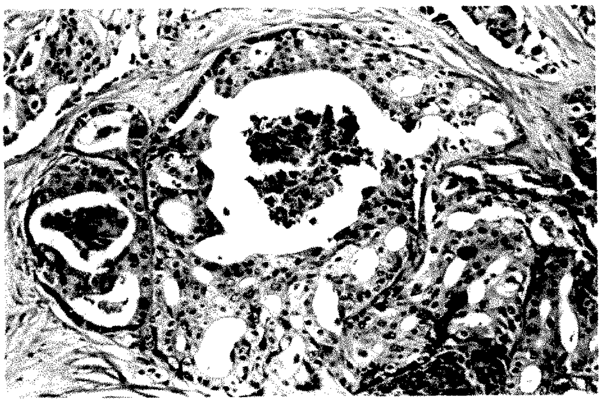

Fig 2. Salivary duct carcinoma (H & E, original x80). Cytoarchitecture of this high-grade carcinoma contrasts with that of terminal duct adenocarcinoma. Cells are larger, are often eosinophilic, and show necrotic debris. These carcinomas have overall resemblance to ductal carcinomas of breast.

terminal duct adenocarcinomas), while those originating from the excretory ducts are high-grade malignancies (eg, salivary duct and squamous cell carcinomas).

The potential for pathologic and, more important, clinical confusion exists because of the terms "duct" and "ductal" in diagnostic descriptions of the carcinomas. Histogenetically and phenotypically correct, these descriptive qualifiers have an ambiguity spawned by the limits of nomenclature. Two prime examples are the clinicopathologic entities terminal duct adenocarcinoma and salivary duct carcinoma (Figs 1 and 2). Each has well-defined site and sex predilections and rather predictable biologic behavior.[3-6]

The purpose of this article is to present the distinctive features of these carcinomas so as to afford a diagnostic reference.

The Table[3-7] (derived from the M. D. Anderson Hospital experience and the literature) compares the two carcinomas. The high-grade salivary duct carcinomas manifest a male predilection and major salivary gland origin, with regional and distant metastasis and death in two thirds of the patients within 5 years. A low-grade biologic behavior and female predilection characterize the terminal duct adenocarcinomas, which are almost exclusively intraoral.

COMPARISON OF TERMINAL DUCT ADENOCARCINOMA AND SALIVARY DUCT CARCINOMA

	Terminal Duct Adenocarcinoma	*Salivary Duct Carcinoma*
No. of patients	69	30
Sex	F 48, M 21	F 7, M 23
Mean age at diagnosis (yr)	49 (23-79)	64 (27-83)
Salivary gland site	Palate 45, buccal mucosa 10, upper lip 7, retromolar 5, others 2	Parotid gland 25, submandibular gland 5
Patients with recurrences	8 (12%)	20 (55%)
Patients with cervical lymph node metastases	7 (10%)	20 (66%)
Patients with distant metastases	0	20 (66%)
Patient deaths attributed to neoplasm	1 (10 yr)	21 (70%) (7 mo to 3 yr)

Data from Batsakis et al,[3] Aberle et al,[4] Frierson et al,[5] Garland et al,[6] and Hui et al.[7]

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COMBINED OTOLARYNGOLOGICAL SPRING MEETINGS

1989 Preliminary Programs

(Subject to change and Council approval)

The Fairmont Hotel, San Francisco

AMERICAN NEUROTOLOGY SOCIETY

Friday, March 31 (all day); Saturday, April 1 (morning)

The William F. House Lectureship: Twenty years of neurotology — my perspective. M. E. Glasscock III, MD

Gadolinium-DTPA enhanced magnetic resonance imaging in acoustic neuroma. M. S. Shapiro, MD; R. K. Jackler, MD; M. J. Lanser, MD

Asymmetric pneumatization of the petrous apex. P. S. Roland, MD; W. L. Meyerhoff, MD, PhD; L. O. Judge, MD

Sigmoid sinus thrombosis diagnosis by contrasted MRI scanning. M. H. Fritsch, MD; T. Wood, MD; K. Koch, MD

Primary intrapetrous meningiomas: current radiographic evaluation and treatment. L. R. Grobman, MD; J. A. Herzog, MD; F. Telischi, MD; T. Naidich, MD

Can gadolinium enhanced MRI replace air cisternography in acoustic tumor diagnosis? G. D. Johnson, MD; L. D. Cromwell, MD; J. E. Freije, MD

Acoustic neuroma removal via retrosigmoid approach 1978-1987. S. G. Harner, MD; C. W. Beatty, MD; M. J. Ebersold, MD

Atypical presentation of acoustic neuromas. A. G. Pfleiderer, MD; J. M. Nedzelski, MD

Acoustic neuroma: a cost-effective approach. M. E. Glasscock III, MD; C. G. Jackson, MD; D. B. Welling, MD; C. I. Woods, MD

ABR in eighth nerve tumor ears: the cross-check principle. A. F. Josey, MS; F. E. Musiek, PhD; M. E. Glasscock III, MD; C. G. Jackson, MD

Specificity of brain stem evoked response audiometry in the diagnosis of retrocochlear hearing loss. M. E. Weiss, MD; D. L. Kisiel, PhD

Canal electrode electrocochleography in patients with absent wave I ABRs. F. E. Musiek, PhD; J. A. Baran, PhD

Intraoperative electrocochleographic monitoring: a guidance analysis system for objective decompression of the hydropic labyrinth in Meniere's disease. I. K. Arenberg, MD; W. P. R. Gibson, MD; L. Best, PhD

The combined pterional-anterolateral approach to cranial base neoplasms. P. G. Smith, MD, PhD; J. P. Leonetti, MD; R. L. Grubb, MD

Subcochlear approach for cholesterol granulomas of the inferior petrous apex. B. Y. Ghorayeb, MD; R. A. Jahrsdoerfer, MD

The perioperative management of the petrous carotid artery in contemporary surgery of the skull base. J. P. Leonetti, MD; P. G. Smith, MD, PhD; R. L. Grubb, MD

Steroid-responsive sensorineural hearing loss in eight patients with posterior fossa masses. C. A. Bowman, MD

Dynamic posturography in bilateral vestibular hypofunction. S. W. Parker, MD

Benign paroxysmal positional vertigo in association with documented first order vestibular dysfunction. R. Y. Fox, MD; J. M. Nedzelski, MD; H. O. Barber, MD

Benign paroxysmal positional vertigo following acute alcohol intoxication. G. B. Brookes, FRCS

Dynamic posturography findings in central nervous system disorders. R. L. Voorhees, MD

Posturography following rotation: a model of posture control during vestibular dysfunction. J. A. Goebel, MD; G. D. Paige, MD

Prone caloric responses in vestibular nerve section patients. M. A. Hamid, PhD; G. B. Hughes, MD; S. E. Kinney, MD

Habituation therapy for chronic vestibular dysfunction. S. A. Telian, MD; N. T. Shepard, PhD; M. Smith-Wheelock, BSME; J. L. Kemink, MD

Vestibulo-ocular reflex responses during electrical (galvanic) stimulation in humans. D. M. Moore, MD; L. Hoffman, PhD; K. Beykirch; V. Honrubia, MD

Use of active high frequency head movements in the diagnosis of Meniere's disease. D. P. O'Leary, PhD; L. L. Davis, RN

The clinical application of acoustic distortion products. L. A. Ohlms, MD; B. L. Lonsbury-Martin, PhD; G. K. Martin, PhD

Chiari-I malformation as a cause of asymmetric sensorineural hearing loss. R. A. Hendrix, MD; A. P. Sclafani; R. DeDio, MD

Diagnosis and treatment of posterior fossa arachnoid cysts. T. J. Haberkamp, MD; W. F. House, MD; E. M. Monsell, MD; L. Piazza, MD; S. C. Levine, MD

Metastatic glomus tumor long-term follow-up. D. I. Bojrab, MD; M. Glasscock III, MD

Bilateral papillary adenocarcinoma of the temporal bone in a patient with von Hippel-Lindau disease. J. F. Kveton, MD

Surgical management of chondroblastoma of the temporal bone. K. L. Horn, MD; H. Hankinson, MD; B. Nagel, MD; M. Erasmus, MD

Consequences of middle ear bone dust during neurotologic surgery. J. G. Faghali, MD; J. E. Benecke, Jr, MD

Otosclerosis involving the vestibular aqueduct and Meniere's disease: a study of human temporal bones. T. H. Yoon, MD; M. M. Paparella, MD; P. A. Schachern

Temporal bone histopathological findings in Alagille's syndrome. H. Takahashi, MD; I. Sando, MD

Glomus tumors — temporal bone histological study. V. S. Dayal, MD; R. Hinojosa, MD; E. Mhoon, MD

New frontiers in otoimmunology. The expression of intermediate filaments in the adult human cochlea. J. E. Veldman, MD, PhD; L. J. J. M. Bauwens, MD; F. C. S. Ramaekers, MD; E. H. Huizing, MD

Three blind cochlear implant patients. R. S. Baker, MD; J. V. D. Hough, MD; N. Wilson, MA; E. Martin, MA

Cochlear implantation in the patient with inner ear malformation. M. A. Novak, MD; T. Balkany, MD; R. Miyamoto, MD; R. C. Fifer, PhD

Histologic evaluation of a human temporal bone with a multichannel cochlear implant. J. K. Niparko, MD; J. J. Zappia, MD; R. A. Altschuler, PhD; J. M. Miller, PhD; D. L. Oviatt, MS; J. L. Kemink, MD

The prognostic value of round window electrical stimulation in cochlear implant patients. S. B. Waltzman, PhD; N. L. Cohen, MD; W. H. Shapiro, MA

Promontory electrical stimulation in labyrinthectomized ears. P. R. Lambert, MD; R. A. Ruth, PhD; C. F. Halpin, PhD

The natural course of labyrinthine ossification following meningitis and its implications for cochlear implantation. M. A. Novak, MD; R. C. Fifer, PhD; J. C. Barkmeier, MD

Trends in rehabilitation following cochlear implantation. D. L. Tucci, MD; P. R. Lambert, MD; R. A. Ruth, PhD

Anatomy of the human cochlear nucleus and the cochlear nerve related to electrical prosthesis placement. L. Terr, PhD; J. Fayad, MD; R. Zakhary, PhD

Investigator differences in the cochlear implantation of children. K. I. Berliner, PhD; W. F. House, MD; H. E. Maddox III, MD

Traumatic intermittent perilymph fistula. S. G. Lesinski, MD; C. S. Whitehurst, MA

SP/AP ratio and perilymph fistula. W. L. Meyerhoff, MD, PhD; W. Yellin, MS

Bilateral spontaneous perilymph fistulae: a diagnostic and management dilemma. A. Sismanis, MD; F. M. Butts, PhD; G. B. Hughes, MD

Multichannel extracochlear implant: advantages and hearing results. J. L. Pulec, MD; M. L. Lewis, MA

Practical management of Bell's palsy. G. B. Hughes, MD

Magnetic evoked facial nerve potentials. J. M. Kartush, MD; K. R. Bouchard, PhD; M. D. Graham, MD

Magnetic transcranial and electric direct stimulation of the facial motor cortex in dogs. S. Estrem, MD; S. Haghighi, PhD, DVM; W. E. Davis, MD

The use of electromyography with electroneurography in the evaluation of facial paralysis. N. J. Coker, MD

A model for evaluating waveform reliability in facial nerve electroneurography. H. N. Gutnick, PhD; R. L. Prass, MD, PhD; M. J. Kelleher, MD

Monitoring facial hyperkinesis during microvascular decompression for hemifacial spasm. R. L. Prass, MD, PhD

Autoimmune hearing loss and central nervous system dysfunction. L. J. Bartels, MD; A. C. Taliaferro, MD

Otologic disease in AIDS patients: CT correlation. D. Kohan, MD; P. Hammerschlag, MD; R. L. Holliday, MD

Estrogen and progesterone binding by acoustic neuroma tissue in vitro. E. W. Monsell, MD, PhD; R. J. Wiet, MD

The high jugular bulb: morphologic features, anatomic relations and implications for neurotologic surgery. S. D. Rauch, MD; W. C. Hsu, MD

Metastatic atrial myxoma to the temporal bone. B. E. Hirsch, MD; D. B. Kamerer, MD; L. Sekhar, MD

Giant cerebellopontine angle vertebral aneurysm. D. A. Chen, MD; J. E. Bailes, MD

An unusual complication of acoustic tumor surgery. A. Kumar, MD; M. Ross, MD; M. Mafee, MD; H. Cramer, MD

AMERICAN LARYNGOLOGICAL ASSOCIATION

Saturday, April 1 (all day); Sunday, April 2 (afternoon)

Endolaryngeal aryenoidectomy in the treatment of bilateral recurrent nerve paralysis. O. Kleinsasser, MD

Botulinum toxin therapy for recurrent laryngeal nerve section failure for adduction laryngeal dystonia. A. Blitzer, DDS, MD; M. Brin, MD; S. Fahn, MD

Reinnervation of the canine posterior cricoarytenoid muscle with preganglionic autonomic neurons. I. N. Jacobs, MD; I. Sanders, MD; B.-L. Wu, MD; H. F. Biller, MD

CO_2 laser cordotomy for treatment of bilateral vocal cord paralysis. H. K. Kashima, MD; D. P. Dennis, MD

Newer technique of laryngeal reinnervation of superior laryngeal nerve (motor branch) as a driver of the posterior cricoarytenoid muscle. A. J. Maniglia, MD; B. Dodds, MD; K. Sorenson, MD; B. Katirji, MD; M. L. Rosenbaum, MA

A Review of the neuroendocrine carcinomas of the larynx. A. Ferlito, MD; I. Friedmann, MD, FRCS

Supraglottic carcinoma: patterns of recurrence. J. T. Johnson, MD; C. M. Lutz, MD; R. L. Wagner; E. N. Myers, MD

Videostroboscopy in the assessment of early glottic carcinoma. R. B. Sessions, MD; S. Miller, MS; G. F. Martin, MD; B. Solomon, MS; L. Harrison, MD; S. Stackpole

Jejunal interposition for repair of stricture or fistula after laryngectomy. G. J. Petruzzelli, MD, PhD; E. N. Myers, MD; E. J. deVries, MD; K. Shestak, MD; J. T. Johnson, MD; R. Wagner

Histology of Isshiki thyroplasty — type I. G. Isaacson, MD; J. C. Kirchner, MD; J. A. Kirchner, MD

Partial laryngectomy after radiation failure. R. D. Nichols, MD

A blinded comparison of office versus operative endoscopy for laryngopharynx cancer staging: toward elimination of preliminary direct laryngoscopy. R. W. Bastian, MD; S. L. Collins, MD; T. K. Kaniff, MD; G. J. Matz, MD

Fat-graft reconstruction of injured canine vocal folds. D. B. Wexler, MD; J. Jiang, MD; S. D. Gray, MD; I. R. Titze, PhD

An improved surgical technique for epidermoid cysts of the vocal fold. M. Hirano, MD; T. Yoshida, MD; J. Hirade, MD; T. Sanada, MD

Complications following rhinotomy: a review of 131 patients. H. F. Biller, MD; W. Lawson, MD, DDS; P. J. Bernard, MD

Laser application to hypertrophic rhinitis. T. Inouye, MD; T. Tanabe, MD; M. Ogura, PhD; M. Nakanoboh, MD; Y. Oomae, MD

A reevaluation of maxillary sinus surgery: an experimental study in rabbits. D. W. Kennedy, MD; H. Shaalan, MD

Calibrated study of sinus anatomy with regard to endoscopic sinus surgery. F. E. Lucente, MD; P. Schoenfeld

Use of nasal valve stent with anterior rhinotomy to quantitate nasal valve obstruction. C. J. Perry, MD; B. J. Guillette, MD

Artificial nasal cycle: a feasibility study in the rat. M. Broniatowski, MD; H. L. Levine, MD; J. C. Kasick; C. R. Davies, MSE; G. B. Jacobs, MSEE; Y. Nose, MD, PhD; H. M. Tucker, MD

Nonsquamous cell carcinoma of the paranasal sinuses. D. H. Kraus, MD; J. K. Roberts, MD; H. L. Levine, MD; B. G. Wood, MD; H. M. Tucker, MD

The apneic technique for laryngoscopy: experience and complications. C. T. Yarington, Jr, MD; B. R. Heckler, MD; W. Martin, MD

Congenital subglottic stenosis: the elliptical cricoid cartilage. L. D. Holinger, MD; R. W. Oppenheimer, MD

Comparison of standard versus prototype endotracheal tube during prolonged endotracheal intubation. P. Santos, MD; A. Afrassiabi, MD; E. A. Weymuller, Jr, MD

A ferret model for acquired subglottic stenosis. K. Alexander, MD; D. E. Schuller, MD

Applications of the voice prosthesis during laryngectomy. M. I. Singer, MD; R. C. Hamaker, MD; E. D. Blom, PhD; G. Yoshida, MD

Voice restoration following laryngopharyngectomy. B. L. Wenig, MD; A. J. Keller, MD; A. L. Abramson, MD

Red blood cell scan in cavernous hemangioma of the larynx. A. M. Noyek, MD; D. Finkelstein, MD; J. C. Kirsh, FRCP

Laryngeal brain stem evoked response. C. K. Aronsen, MD; L. Lalakea, MD

Regional blood flow to the canine vocal fold at rest and during phonation. D. P. Arnstein, MD; G. S. Berke, MD; T. K. Trapp, MD; M. Natividad

Proliferation and differentiation characteristics of normal human squamous mucosal cells of the upper aerodigestive tract. J. L. Kasperbauer, MD; B. M. Hsu; H. B. Neel III, MD, PhD; R. E. Scott, MD

The laryngeal manual compression test. S. M. Blaugrund, MD; T. Taira, MD; A. El-Assuooty, MSEE; W. J. Gould, MD; N. Isshiki, MD

Bolus flow characteristics during normal deglutition. R. J. Nelson, MD; R. H. Mathog, MD

The role of the soft palate in the voice quality and laryngeal functions — a videoendoscopic study. E. Yanagisawa, MD; S. Kmucha, MD; J. Estill

Clinical measure of laryngeal efficiency. R. J. Lipton, MD; D. H. Slavit, MD; T. V. McCaffrey, MD, PhD

The subglottic larynx. J. A. Tucker, MD

AMERICAN OTOLOGICAL SOCIETY, INC
Sunday, April 2 and Monday, April 3 (mornings)

Magnetic resonance: 1988 update. G. E. Valvassori, MD

Clinical management of the bilateral acoustic neurofibromatosis (NF-2) patient. C. Monaco, MS; G. J. Matz, MD; D. Benedek, MA; J. Mullan, MD

Possible precursors of regenerated hair cells in the avian cochlea following acoustic trauma. E. W. Rubel, PhD; D. Girod, MD; L. Duckert, MD, PhD

Recent advances in inner and outer hair cell functions. D. J. Lim, MD

A therapeutic trial of nortriptyline in patients with tinnitus and major depression. M. D. Sullivan; W. J. Katon; R. A. Dobie, MD; C. S. Sakai; J. Russo

Auditory monitoring of ototoxic drugs. W. L. Meyerhoff, MD, PhD; M. W. Yellin, MS, CCC/A; G. E. Maale, MD; P. S. Roland, MD

Use of ceftazidime in the treatment of malignant external otitis. C. P. Kimmelman, MD; F. E. Lucente, MD

Oral antibiotic therapy for malignant external otitis. J. Rubin, MD; V. L. Yu, MD; D. B. Kamerer, MD

Thickness of the lateral surface of the temporal bone in children. D. Simms, MD; J. G. Neely, MD

Open-set speech recognition by children with a single-channel cochlear implant. K. I. Berliner, PhD; W. F. House, MD; L. L. Tonokawa, MS

Comparison of sensory aids in deaf children. R. T. Miyamoto, MD; A. J. Robbins, MS; J. Renshaw, MA; M. J. Osberger, PhD; W. A. Myres, MAT: K. Kessler, MS; M. L. Pope, MAT

Telephone speech comprehension using the Nucleus cochlear implant. N. L. Cohen, MD; S. B. Waltzman, PhD; W. H. Shapiro, MA

Cochlear implant revision: the effects of explantation followed by reimplantation. R. K. Jackler, MD; P. A. Leake, PhD; W. McKerrow, FRCS; H. A. Milczuk, MD

Results following reimplantation of cochlear implants. B. J. Gantz, MD; B. F. McCabe, MD

Effects of unilateral loss of vestibular function on vestibulo-ocular reflex and postural control. F. O. Black, MD; C. L. Shupert; R. J. Peterka; L. M. Nashner

Sudden hearing loss due to perilymphatic fistulae: a single-blind temporal bone histopathologic study. R. I. Kohut, MD; R. Hinojosa, MD; J. H. Ryu, PhD

Otoscopy for the diagnosis of otologic disease. R. L. Goode, MD; K. Nakamura, MD

Meniere's syndrome and endolymphatic hydrops — a double-blind temporal bone study. S. D. Rauch, MD; S. Merchant, MD

Long-term results with sacculotomy for Meniere's disease. G. D. L. Smyth, MD

Vestibular dysfunction associated with otosclerosis. J. R. Emmett, MD; J. J. Shea, MD

Assessment of infants with aural atresia using auditory brain stem response to bone-conducted tones. D. R. Stapells, PhD; R. J. Ruben, MD

Treacher Collins syndrome: an otologic challenge. R. A. Jahrsdoerfer, MD; E. A. Aquilar, MD; J. W. Yeakley, MD; R. R. Cole, MD

Spontaneous cerebrospinal fluid otorrhea. G. H. Conner, PhD

Complexities of lateral skull base surgery. I. P. Janecka, MD; L. N. Sekhar, MD

Cadaver middle ears as models for living ears. J. J. Rosowski, PhD; P. J. Davis; S. N. Merchant, MD; K. M. Donahue; M. D. Coltrera, MD

Ceravital versus Plastipore ossicular replacement prostheses. C. A. Mangham, MD; R. C. Lindeman, MD

Recurring cholesteatomas in children, adolescents and adults. A. G. Schuring, MD; W. H. Lippy, MD; F. M. Rizer, MD; L. Schuring, MSN, RN

Cholesteatoma involving the petrous pyramid with posterior or middle cranial fossa extension. A. J. Maniglia, MD

AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

Monday, April 3 and Tuesday, April 4 (afternoons)

Chronic pediatric stridor: an analysis of 60 cases. E. M. Friedman, MD; P. Vastola; T. McGill, MD; G. B. Healy, MD

Rapid acquisition computerized tomography (cine-CT) in the evaluation of upper and lower airway obstruction in infants and children. D. M. Crockett, MD; E. E. Frey, MD; W. Stanford, MD; J. S. Wagener, MD

Avoidance of tracheotomy in infants with significant subglottic stenosis. L. A. Halstead, MD

Selected use of laser dilation and stenting for complex cervical tracheal stenosis. S. M. Shapshay, MD; J. F. Beamis, Jr, MD

The quantitative study of the early effects of tracheotomy and endotracheal intubation on the rabbit tracheobronchial tree. R. Squire, MD; S. Siddiqui, MD; G. Dinunzio, MD; L. Brodsky, MD

The effect of laryngeal stenting in a rabbit model. D. M. Albert, MD; R. T. Cotton, MD; P. Conn, MRC

Oral injuries due to prolonged endotracheal intubation. R. Cusumano, MD; P. Bhatia, MD; Y. P. Krespi, MD

Laryngeal granulomatous polyp after short-term intubation of a child. D. Drosnes, MD; D. Zwillenberg, MD

Posttracheotomy suprastomal granulation managed by carbon dioxide laser excision. J. Werkhaven, MD; B. R. Maddern, MD; S. E. Stool, MD

Chronic cough — look for hiatus hernia and reflux. M. I. Toubbeh, MD; P. Kandalaft, MD; A. P. Wolff, MD

Protracted dysphagia caused by esophageal impingement with erosion. R. G. Farr, MD; R. W. Babin, MD

Cricopharyngeal myotomy: indications and technique. J. McKenna, MD; H. H. Dedo, MD

Complications of primary tracheoesophageal fistula following total laryngectomy. D. T. Jones, MD; F. P. Ogren, MD; W. Stiles, MD

Endoscopic diathermy obliteration of recurrent tracheoesophageal fistulae. C. Griffiths, MD; D. Zwillenberg, MD; P. Wolfson, MD

Laryngotracheoesophageal cleft: role of early surgical repair. C. M. Myer III, MD; R. T. Cotton, MD; R. K. Jackson, MD

Prevention of aspiration in infants and children — federal regulations. J. S. Reilly, MD

A new set of microlaryngeal instruments for phonosurgery and pediatric applications. S. M. Shapshay, MD; G. B. Healy, MD

Use of a molar dental band to secure the proximal end of a guide string for esophageal dilatation. L. J. Dindzan, MD; J. Werkhaven, MD; S. E. Stool, MD

Laser surgery to carcinoma of larynx — the second report. T. Inouye, MD; T. Tanabe, MD; M. Ogura, MD

Frontal sinus osteoma. M. E. Smith, MD; T. C. Calcaterra, MD

The effect of systemic bleomycin on the treatment of solid tumors with hematoporphyrin derivative phototherapy. G. C. Jones, MD; H. B. Neel III, MD, PhD; D. A. Cortese, MD

The importance of iron repletion in the management of the Plummer-Vinson syndrome. J. K. Bredenkamp, MD; D. J. Castro, MD; R. A. Mickel, MD, PhD

Laryngeal amyloidosis in a child — a case report. C. G. Hurbis, MD; L. D. Holinger, MD

Severe otolaryngological manifestations of arthrogryposis multiplex congenita. L. P. Rybak, MD, PhD; A. Laureano, MD

Laryngeal manifestations of gout. M. D. Guttenplan, MD; M. J. Townsend, MD; G. Balsara, MD; S. E. Ladenheim, MD

Human papillomavirus in lesions of the oral cavity: a study of carcinoma, leukoplakia, lichen planus and clinically normal epithelial specimens. H. Kashima, MD; T. Kessis, MD; M. Kutcher, MD; L. S. Levin, MD; E. M. Devilliers, MD; K. Shah, MD

Pulmonary parameters in children after laryngotracheal reconstruction. J. Thomsen, MD; C. Derkay, MD; H. Chaney, MD; G. Zalzal, MD

Failure of physiologic parameters as predictors of acute airway obstruction. D. W. Eisele, MD; M. D. Coltrera, MD; D. A. Bright, MD; E. A. Weymuller, Jr, MD

Delayed airway obstruction and neck abscess following manual strangulation injury. D. B. Kuriloff, MD; R. Pincus, MD

Hydroxyl apatite for laryngotracheal framework reconstruction. M. Hirano, MD; T. Yoshida, MD; S. Sakaguchi, MD

Management of neonatal cervical masses with airway obstruction. M. F. Kelly, MD; L. Berenholz, MD; D. Zwillenberg, MD

Bronchogenic cyst with tracheal involvement: considerations in management. H. M. Yerman, MD; A. K. Bowes, MD; L. D. Holinger, MD

Second pharyngeal pouch cysts. C. Drucker, MD; M. Mikhael, MD; A. P. Wolff, MD

Glottographic analysis of phonation in an excised canine larynx. D. H. Slavit, MD; R. J. Lipton, MD; T. V. McCaffrey, MD

Risk factors of vocal cord hemorrhage. P.-T. Lin, MD; W. J. Gould, MD

AMERICAN LARYNGOLOGICAL, RHINOLOGICAL AND OTOLOGICAL SOCIETY, INC

Tuesday to Thursday, April 4 to 6 (mornings)

Prognostic validity of BERA in infants. P. W. Alberti, MB, PhD; M. L. Hyde, PhD; K. Riko, MSc

The electrical auditory brain stem response evaluates auditory nerve survival. R. C. Fifer, PhD; M. A. Novak, MD

Same-day ear molds and hearing aids in a medical school setting. C. I. Berlin, PhD; G. D. Lyons, MD; H. Berry

A digitally programmable hearing system with multiband compression and ultrasonic remote control. R. Perkins, MD; F. Waldhauer; V. Pluvinage, PhD; A. J. Becker; C. Baez

Lightning and its effects on the auditory system. D. T. Jones, MD; F. P. Ogren, MD; L. Roh, MD

Objective tinnitus in benign intracranial hypertension: an update. A. Sismanis, MD; F. M. Butts, PhD; G. B. Hughes, MD

Composite autograft for tympanoplasty and tympanomastoid surgery. W. Y. Adkins, MD

Congenital absence of the oval window. P. R. Lambert, MD

The enlarged vestibular aqueduct syndrome. R. K. Jackler, MD; A. De La Cruz, MD

Portable vestibulo-ocular reflex test in ototoxic patients. M. Fortin, MD; J. A. Goebel, MD; G. D. Paige, PhD, MD

Strategies in vestibular rehabilitation. D. B. Kamerer, MD; J. M. R. Furman, MD, PhD; B. E. Hirsch, MD

Magnetic stimulation of the facial nerve in humans. S. A. Estrem, MD; R. R. Conway, MD; W. Platt, MD; S. Haghighi, PhD; W. Davis, MD; J. Oro, MD

Cranial polyneuropathy in herpes zoster. L. I. Golden, MD; Z. E. Deeb, MD; H. O. deFries, MD

Reconstruction of base of skull surgical defects. I. P. Janecka, MD; L. N. Sekhar, MD; C. H. Snyderman, MD; S. Ramasastry, MD

Extracranial meningiomas of the head and neck: diagnosis and management. C. D. Friedman, MD; P. Costantino, MD; R. Berktold, MD; G. A. Sisson, MD

The use of fibrin glue in intracranial procedures: preliminary results. D. Sierra, MSBE; A. J. Nissen, MD; J. Welsh, MD

Control of bleeding in extended skull base surgery. J. P. Leonetti, MD; P. G. Smith, MD, PhD; R. L. Grubb, MD

Effects of preoperative embolization of glomus jugulare tumors. T. Murphy, MD, MAJ, USA; D. E. Brackmann, MD

Long-term results following glomus jugulare surgery. C. G. Jackson, MD; D. S. Poe, MD; G. D. Johnson, MD; M. E. Glasscock III, MD

Electroglottographic voice analysis of patients following near-total laryngectomy and total laryngectomy with tracheoesophageal puncture. T. McCaffrey, MD; R. J. Lipton, MD; K. D. Olsen, MD; B. W. Pearson, MD

Update of the Cincinnati experience of pediatric laryngotracheoplasty: 270 cases. R. T. Cotton, MD; R. P. Miller, MD; S. Gray, MD

Modifications of epiglottic laryngoplasty reconstruction for larynx cancer. R. W. Bastian, MD; S. L. Collins, MD

Management of the divided recurrent laryngeal nerve. D. Green, MD; P. H. Ward, MD; D. Hanson, MD

Respiratory activity of the cricothyroid muscle in conscious humans. G. E. Woodson, MD

Endoscopic management of frontal sinus disease. S. D. Schaefer, MD; L. G. Close, MD

Transethmoid-sphenoid optic nerve decompression. M. P. Joseph, MD; S. Lessell, MD; J. Rizzo, MD

Physiologic control of nasal airway resistance. T. McCaffrey, MD

Melanoma of the nasal cavity. J. Conley, MD

Management of inverted papilloma: an analysis of 70 cases. J. Le Benger, MD; W. Lawson, MD; P. Som, MD; H. F. Biller, MD

Proton nuclear magnetic resonance spectroscopy of plasma lipoproteins in head and neck cancer patients. J. M. Chow, MD; E. D. Gotsis, PhD; M. Jarc, MD; J. Eiesenga, MD; A. M. Wyrwicz, PhD; E. L. Applebaum, MD

Lyphokine-activated killing of autologous and allogeneic short-term–cultured head and neck squamous carcinoma. F. R. Leess, DDS, MD; R. Mickel, MD, PhD; A. Lichtenstein, MD

Rationale for prophylactic neck dissection — 1988. M. Freidman, MD; M. F. Mafee, MD; V. T. Grybauskas, MD

Parathyroidectomy: a review of 52 patients. D. W. Stein, MD; R. Hamaker, MD; S. Freeman, MD

Upper airway measurements predict response to uvulopalatopharyngoplasty. R. I. Dickson, MD; C. F. Ryan, MB, MRCPI; A. A. Lowe, DMD, PhD; A. Blokmanis, MD; J. A. Fleetham, MD

Perioperative complications and risk factors in the treatment of obstructive sleep apnea syndrome. R. M. Esclamado, MD; M. G. Glenn, MD; T. M. McCulloch, MD; C. W. Cummings, MD

Combined craniofacial resection for base of skull neoplasms: the Cleveland clinic experience. T. L. Good, MD; D. E. Wood, MD; H. L. Levine, MD; B. G. Wood, MD

The rectus abdominus free flap in head and neck reconstruction. R. E. Hayden, MD

The innervated radial forearm free flap in head and neck reconstruction. M. L. Urken, MD; H. Weinberg, MD; C. Vickery, MD; H. F. Biller, MD

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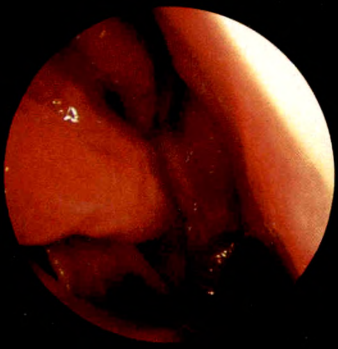

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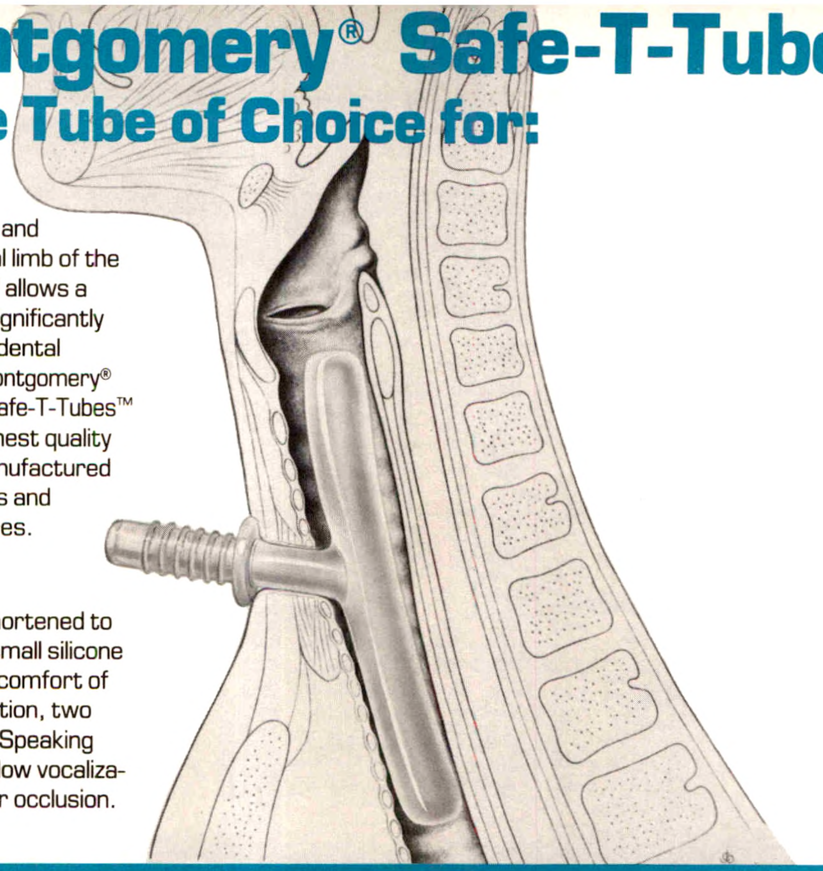

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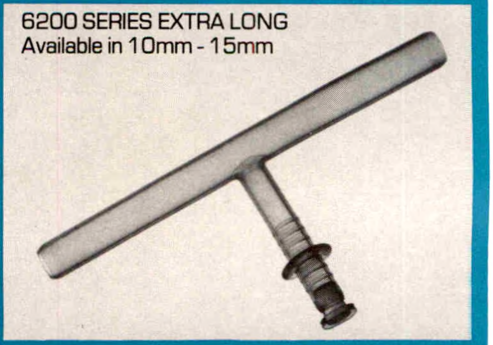

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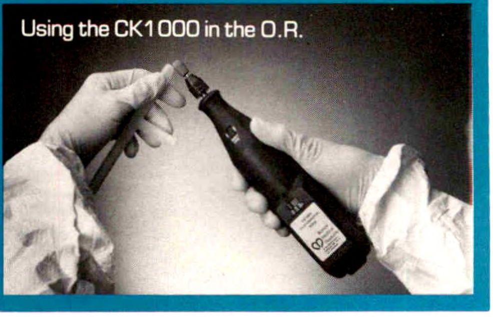

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BLOOD TRANSFUSIONS AND RECURRENCE IN HEAD AND NECK CANCER

ROBERT M. JACKSON, MD DALE H. RICE, MD

LOS ANGELES, CALIFORNIA

Recent reports in the general surgery literature have shown decreased survival with blood transfusions in abdominal cancer surgery. We reviewed our experience with 100 consecutive patients, all of whom had 5-year follow-up, to see if this held true for head and neck cancer. The detailed report covers site and stage. In summary, however, the recurrence rate for all cancers of the larynx was 14% for those who did not receive blood and 65% for those who did. For cancer of the oral cavity, pharynx, and nose or sinus, the recurrence rate was 31% without transfusions and 71% with transfusions. It would appear that the use of blood transfusions somehow adversely affects the patient's survival. Further study needs to be done to elucidate the mechanism.

KEY WORDS — blood transfusion, head and neck cancer.

INTRODUCTION

The depression of cellular immunity in head and neck cancer patients has been well documented.[1-3] Surgical and radiation therapy have both been shown to suppress immunologic function as well.[4,5] Recent reports suggest that blood transfusions potentiate the immunosuppression seen with surgical treatment.[6] In addition, several studies have shown increased tumor recurrence with perioperative transfusion of blood products in patients with cancer of the colon,[7] lung,[8] breast,[9] and extremities.[10] To our knowledge, however, there have been no reports on the effect of blood transfusion on recurrence in head and neck cancer patients. We therefore reviewed our experience with 100 consecutive patients who received surgical treatment of their head and neck cancer at the University of Southern California-affiliated hospitals.

PATIENTS AND METHODS

The records of 100 consecutive patients diagnosed as having head and neck cancer were reviewed. Only patients treated surgically for cure and (except for stage I lesions) with postoperative radiation therapy were included. Patients who did not have clear surgical margins (confirmed by the final pathologic examination) were omitted from the study. Complete transfusion records and 5-year follow-up data were available in all patients. Patients were staged according to the standards set by AJC in 1980.[11]

The age range was from 31 to 83 years (average, 59 years). There were 75 men and 25 women.

All patients had biopsy-proven squamous cell carcinoma prior to definitive treatment. The most common site of cancer was the larynx. Other sites included in this study were the oral cavity, oropharynx, hypopharynx, intranasal area, and maxillary sinus. Skin, lip, parotid, and thyroid tumors were omitted, as transfusions are rare with these tumors.

RESULTS

The 100 patients were divided into two groups according to site. Group 1 included the patients with laryngeal cancer, while group 2 included patients with cancer of the oral cavity, pharynx, and nose or sinus. Sixty patients had cancer of the larynx, while 40 patients were in the mixed-site group (the majority of the latter having cancer of the oral cavity or pharynx). Each group was then divided into those who had received transfusion of blood products (packed red blood cells or whole blood) either during surgical treatment or within 72 hours postoperatively. No patient received transfusion after this time period. Recurrence rates were calculated within both groups by individual stage, as well as for total patients.

In group 1 (laryngeal cancer), the overall recurrence rate of patients receiving blood transfusion was 65% as compared to 14% for the patients without transfusion (see Table). This is significant at $p < .001$. By stage, the recurrence rates with versus without transfusion, respectively, were as follows: stage II, 100% versus 14%; stage III, 80% versus 13%; and stage IV, 55% versus 33%. No stage I patients received a transfusion. Thus, in terms of both individual staging and the total group, recurrence rates for laryngeal cancer were higher for those who received perioperative blood transfusion.

In group 2 (see Table; oral cavity, pharynx, and nose or sinus), the recurrence rates with versus without transfusion were as follows: stage I, 100% versus 0%; stage II, 80% versus 17%; stage III, 56% versus 67%; and stage IV, 75% versus 100%.

From the Department of Otolaryngology-Head and Neck Surgery, University of Southern California, and the Los Angeles County-University of Southern California Medical Center, Los Angeles.

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RELATION BETWEEN STAGE AND RECURRENCE

	Stage				
	I	*II*	*III*	*IV*	*Total*
Laryngeal cancer (n = 60)					
With transfusion	—	1/ 1 (100%)	4/ 5 (80%)	6/11 (55%)	11/17 (65%)
Without transfusion	0/3 (0%)	3/21 (14%)	2/16 (13%)	1/ 3 (33%)	6/43 (14%)
Cancer of oral cavity, pharynx, nose or maxillary sinus (n = 40)					
With transfusion	2/2 (100%)	4/ 5 (80%)	5/ 9 (56%)	6/ 8 (75%)	17/24 (71%)
Without transfusion	0/5 (0%)	1/ 6 (17%)	2/ 3 (67%)	2/ 2 (100%)	5/16 (31%)
Cancer of all sites (n = 100)					
With transfusion	2/2 (100%)	5/ 6 (87%)	9/14 (64%)	12/19 (63%)	28/41 (69%)
Without transfusion	0/8 (0%)	4/27 (15%)	4/19 (21%)	3/ 5 (60%)	11/59 (19%)

Figures are numbers of patients with cancer recurrence per total numbers of patients.

For all patients in this group, the recurrence rates were 71% versus 31%, respectively. This is significant at $p<.02$. Although the total number of patients and those with stage I and II disease were consistent with higher recurrence rates with transfusion, the rate was lower for those with stage III and IV disease, but patients with stage III and IV disease not receiving transfusions were too few to test for significance.

Lastly, for all 100 patients (see Table), the recurrence rates with versus without transfusion were as follows: stage I, 100% versus 0%; stage II, 87% versus 15%; stage III, 64% versus 21%; and stage IV, 63% versus 60%. For all patients, the recurrence rates were 69% versus 19%, respectively. This is significant at $p<.001$. Thus, when all sites were included, the recurrence rates with transfusion were significantly higher in the total number of patients, as well as stage for stage (except for stage IV, for which the rates are similar, but the number of patients not receiving transfusion was too small to test for significance). All failures in these 100 patients were locoregional.

DISCUSSION

The depressed immune system of the head and neck cancer patient is an accepted prognostic indicator of future tumor recurrence. Deficits include delayed cutaneous hypersensitivity,[1] diminished lymphocyte proliferation,[2] and a decreased interleukin-2–producing subset of T helper-inducer cells.[3] Many variables that may adversely affect tumor recurrence rates have been studied. In patients with squamous cell cancer of the head and neck, surgery has been shown to diminish natural killer cell activity, which has been correlated with the ability to eliminate blood-borne embolic metastases.[4] Gray et al[5] studied the depressed cellular immunity in head and neck cancer patients by measuring total lymphocyte levels, T and B cell fractions, and the B/T cell ratio. All parameters decreased postoperatively, although only for a period of up to 2 weeks. However, they found that radiation therapy depressed all of these measures up to 11 years afterward; this finding may have significance for recurrence and survival rates. Waymack et al[6] investigated the effect of transfusion on the immune system in an animal study. They found that transfusions resulted in impaired cell-mediated immunity and macrophage migration, without diminishing the white blood cell count, differential cell count, or neutrophil migration and bactericidal index.

The effect of blood transfusion on cancer recurrence has been studied in colorectal carcinoma.[7] In Duke's stages A, B1, and B2 (as cited by Blumberg et al[7]), the recurrence rates with and without transfusion were 41% and 7%, respectively. In the more advanced colorectal cancers (stage C), the recurrence rates were 54% with transfusion and 14% without transfusion. Patients undergoing surgical resection of stage I (subset N0) non–oat cell lung cancer were found to have a lower 5-year disease-free rate if they received blood transfusion than if they did not (62% versus 76%).[8] Similar results have been reported with breast cancer (51% 5-year disease-free survival rate with transfusion, compared to 65% without transfusion).[9] In addition, the 5-year survival has been found to be decreased in patients with high-grade soft tissue sarcomas of the extremities who receive transfusion (85% versus 63%).[10]

To our knowledge, there have been no prior reports on the effect of blood transfusion on recurrence rates in head and neck cancer patients. In our study, we found higher recurrence rates for laryngeal cancer in the patients who received transfusion. The argument that larger tumors, with an inherently poorer prognosis, would more likely be accompanied by transfusion is not applicable, since the recurrence rates were higher in each individual clinical stage of the disease. Because all resections were performed by the same two attending surgeons (although with residents), technique-related variables were minimized and the criteria for when to transfuse were identical.

In the group of patients with oral, pharyngeal, and nose or sinus cancer, the earlier stages (I and II) had a greater chance of recurrence with transfusion. The stage III patients had approximately the same chance with or without transfusion, and the stage IV patients had a somewhat lesser chance of recurrence with transfusion. This may reflect the poor prognosis that exists in larger tumors in these sites, which would be affected less by variables such as blood transfusion, but probably reflects the small number not receiving blood.

CONCLUSION

One hundred patients with head and neck cancer treated for cure either surgically or with combined (surgical and radiation) therapy were reviewed. Recurrence rates were uniformly higher for patients who received perioperative blood transfusion. Whether or not this association is causative is unknown, but it does imply that blood transfusion adversely affects the patient with head and neck cancer, as has been similarly shown in cancer of the colon, breast, lung, and extremities.

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We present a series of 21 children with obstructive sleep apnea syndrome and sickle cell disease (SCD). Thirteen patients underwent adenotonsillectomy with resolution of symptoms and improvement in alveolar hypoventilation. Adenotonsillectomy involves significantly increased risks in patients with SCD. We present our protocol for management of these difficult surgical patients, including vigorous hydration and transfusion therapy. Polysomnography has proven valuable both in diagnosis and in identifying severely affected patients. Increased end tidal alveolar carbon dioxide during sleep has proven to be a significant predictor of disease, while oxygen saturation monitoring was shown to be unreliable. The incidence of adenotonsillar hypertrophy (ATH) in SCD appears increased and not related to infectious diseases. We suggest that ATH represents a part of the natural course of compensatory lymphoid tissue enlargement in children with SCD.

KEY WORDS — children, obstructive sleep apnea, sickle cell disease.

INTRODUCTION

Since the original description of obstructive sleep apnea syndrome (OSAS) in 1965,[1] limited work has focused on its various causes in children. Upper airway obstruction secondary to adenotonsillar hypertrophy (ATH) has been recognized as a significant cause of sleep disturbance, cor pulmonale, and failure to thrive.[2-4] In children with sickle cell disease (SCD), the superimposition of OSAS upon the underlying hemoglobinopathy gives rise to increased morbidity. This is believed to be due to the hypoxemia, hypercarbia, and acidosis of OSAS—conditions that tend to favor increased sickling.

Obstructive sleep apnea syndrome secondary to ATH has been reported in only two patients with SCD.[5] The cause of ATH in SCD is unclear. Several mechanisms have been proposed, including compensatory hypertrophy of lymphoid tissue in response to functional splenectomy, reactive enlargement due to repeated infections with encapsulated organisms, and increased hematopoietic needs because of the chronic hemolytic anemia.

Sickle cell disease is an autosomal inherited hemoglobinopathy that largely affects people of African descent, but also those of Mediterranean and Indian descent. It is characterized by a hemolytic anemia and vaso-occlusive complications due to the production of the abnormal hemoglobin. The benign heterozygous state, sickle cell trait, affects approximately 8% to 10% of the black American population, while SCD (hemoglobin SS) is found in one of 500 black newborns. The estimated incidence of SCD in the black population is 0.5%.[6]

The molecular, physiochemical, and pathophysiologic basis of SCD has been reviewed extensively in several publications.[6] Our interest in OSAS and SCD began with our overall interest in sleep disturbances in children. Because of our large SCD population, we began to notice children with classic symptoms of OSAS along with SCD. The use of sleep studies has been of great help in the detection of patients afflicted with OSAS.

Surgery in SCD patients carries a significantly increased risk due to anemia, the risk of hypoxia, and postoperative complications. Changes in P_{CO_2}, P_{O_2}, temperature, or acid-base status affect sickling and may precipitate a vaso-occlusive crisis or ischemia.

We outline a treatment protocol including preoperative transfusion therapy and postoperative care of these patients.

MATERIALS AND METHODS

Our patient population is from the Sickle Cell Center of Southern Louisiana based at Charity Hospital and Tulane Medical Center. This comprehensive sickle cell program cares for approximately 400 children and adolescents with SCD.

Patients presented with a classic history, symptoms, and physical examination results consistent with OSAS,[7,8] which is defined as constant snoring with retractions and obstructive apnea pauses associated with significant alveolar hypoventilation resulting in decreased O_2 saturation and hypercarbia during sleep. Twenty-one patients 2 to 21 years of age with SCD underwent polysomnography between 1983 and 1986. Sleep studies were employed

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with the same criteria as for the general population.[9] Polysomnography was conducted in an approved sleep laboratory as either a nap or overnight study. Recordings included ECG, alveolar CO2 (PACO2), transcutaneous O2 or O2 saturation, impedance pneumography, and transnasal airflow measurements. Normal polysomnographic data are not available on children with SCD to serve as age- and sex-matched controls, so that preoperative and postoperative data were collected using our patients as their own controls.

Obstructive sleep apnea syndrome was diagnosed on the basis of a sleep study with a consistent clinical history and physical examination results. All patients who met the criteria for OSAS were referred for tonsillectomy and adenoidectomy.

TREATMENT PROTOCOL

Preoperative evaluation and transfusion therapy were coordinated by the pediatric hematologist. Chest and sinus radiographs, CBC, reticulocyte count, electrolytes, coagulation studies, liver functions, and quantitative hemoglobin electrophoresis were obtained on all patients.

Over a 2- to 3-week period, patients were transfused with washed packed RBCs to bring the preoperative hematocrit to a minimum of 35% and the ratio of hemoglobin A to hemoglobin S to at least 60:40. A hemoglobin electrophoresis was repeated the morning of operation to ensure adequate hemoglobin A levels.

One or 2 days prior to operation patients were admitted for final transfusion, hydration, and hemoglobin electrophoresis. Intravenous (IV) fluid therapy was begun at one or $1\frac{1}{2}$ times maintenance needs to ensure adequate hydration during the period of restricted oral intake. Penicillin prophylaxis was changed from oral to IV and maintained throughout the hospitalization.

Anesthetic management of each patient was performed by anesthesiologists in accordance with the surgeon and pediatric staff. Atropine or glycopyrrolate alone was used as a preoperative medication. Sedatives were not used because of the risk of respiratory depression and the potential for O2 desaturation. Standard agents were used for induction and maintenance of anesthesia and a warming blanket was used as needed.

A tonsillectomy and adenoidectomy was performed with dissection and snare technique. Bovie cautery and packing were used for hemostasis, following which patients were suctioned meticulously prior to extubation.

Postoperatively, IV fluid and O2 therapy were continued until patients were ambulatory and tolerating fluids. Pain medications and sedation were tapered specifically to each patient's needs. Patients were monitored carefully to achieve a careful balance between adequate pain relief and possible respiratory depression. Steroids were not used.

Postoperative polysomnograms were obtained whenever possible, usually between 6 weeks and 6 months.

Growth and development data are kept routinely by the sickle cell center. Height, weight, and growth percentiles were obtained from patient charts when complete data were available. The data were analyzed statistically using a paired Student's *t* test.

RESULTS

A total of 21 patients with SCD (Hgb SS) underwent evaluation for OSAS. Based on history, physical examination, and sleep study all patients met the criteria for OSAS. Thirteen patients (11 female and two male) underwent surgery. Four patients declined surgery and four were not available for follow-up and had no surgery. The mean age for all patients was 8.75 years (range, 22 months to 21 years).

All children who had adenotonsillectomy improved dramatically both in subjective complaints (snoring, sleep disturbance, restlessness, and school attendance and performance) and objectively in sleep study parameters. The results of end tidal CO2 during the sleep studies are summarized in the Table. End tidal PACO2 during sleep decreased an average of 12 mm Hg (range, 3.5 to 25 mm Hg) following adenotonsillectomy. This difference was statistically significant ($p < .05$). The mean number of apneas was 7.2 episodes per hour (range, three to 20) in the surgery group. The length of apnea ranged from 10 to 25 seconds. All apneas longer than 10 seconds resolved with surgery. There was no statistically significant difference in number of apneas between the preoperative and postoperative or surgery and no-surgery groups, however.

Oxygen saturation data changes were not significant when comparing either arterial O2 saturation or transcutaneous O2 for different levels of decreasing oxygenation currently accepted as indicative of hypoxemia (O2 saturation decrease of 4% or transcutaneous O2 change of 15 mm Hg).

The most consistent indication for surgery and best predictor of response was increased end tidal PACO2 during sleep (greater than 50 mm Hg) and obstructive apneas longer than 15 seconds.

Three of four patients who did not have surgery but had a follow-up sleep study improved spontaneously, while one had a fluctuating course followed by worsening of sleep study results.

Surgery was conducted without any complications and there was no morbidity or mortality in our

END TIDAL ALVEOLAR CARBON DIOXIDE MEASUREMENTS

	Surgery Group (n = 13)		*No-surgery Group (n = 4) (Follow-up > 1 Year)*	
	Preoperative	*Postoperative*	*Initial*	*Follow-up*
Mean awake P_{ACO2} (mm Hg)	50 (43-62)	40 (33-50)	46 (40-54)	40 (33-44)
Mean asleep P_{ACO2} (mm Hg)	54 (43-62)	42 (35-54)*	48 (43-56)	42 (35-47)

Range of values in parenthesis.

*Difference between preoperative and postoperative asleep P_{ACO2} was significant ($p < .05$).

series. Transfusion therapy was used in all patients perioperatively to relieve the burden of hemoglobin S and to provide additional O_2-carrying capacity. The average pretransfusion hemoglobin level was 7.85 g/dL and the average posttransfusion (preoperative) level was 12.3 g/dL. The average operative blood loss was 4 mL/kg.

According to our protocol, patients were admitted preoperatively for 1 or 2 days of IV hydration and final transfusion therapy if indicated. Patients averaged 3 days of postoperative care for IV hydration and to ensure adequate oral intake.

We analyzed growth and development data from available patients, as these data are kept routinely by the sickle cell clinic. These patients are often growth-delayed by one or two standard deviations. We constructed a weight gain velocity comparison chart based on percentile weight and using time of surgery or sleep study as a point of reference (see Figure). Weight curve velocity appeared to improve markedly in most patients following adenotonsillectomy, while the no-surgery group failed to show significant gains. However, not enough growth data from a sufficient number of patients were generated to demonstrate statistically significant changes in growth.

The incidence and prevalence of OSAS in SCD is unreported. Based on our population, a period prevalence of 5% can be calculated.

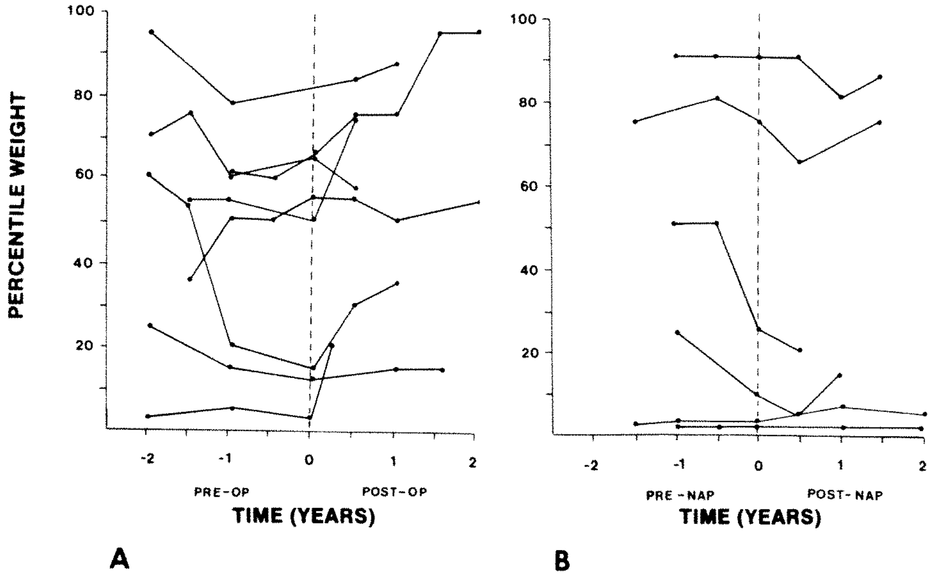
DISCUSSION

Surgery in SCD patients has substantial risk.[10-12] Extensive workup and coordinated care are required to avoid the potential morbidity and mortality. Surgery can be performed relatively safely in these patients although earlier studies have quoted up to 50% morbidity and 10% mortality in large series—including a death following tonsillectomy.[13,14]

We stress a conservative management plan and a coordinated effort between the pediatric hematologist, anesthesiologist, and otolaryngologist.

Perioperative complications are due to sickling, and care should stress maintenance of several important physiologic factors. Postoperative pulmonary care, including O_2 therapy, is important in order to avoid hypoxemia and the risk of sickling. Adequate hydration to maintain above-normal blood flow through end capillary systems is stressed and may help prevent vaso-occlusive crisis. Limitation in the use of narcotics is essential to avoid hypoxia, hypercarbia, and acidosis secondary to hypoventilation. We routinely avoid any preoperative medications except glycopyrrolate or atropine. Lastly, thermoregulation and avoidance of cold stress during surgery is important in preventing possible postoperative pain crisis.

Transfusion therapy for perioperative management of SCD patients was begun several years ago



Weight change A) in surgery group following adenotonsillectomy (at time 0) and B) in no-surgery group over study period (with time 0 being time of initial nap study).

and has altered dramatically previous morbidity and mortality.[15] We used transfusion or exchange transfusion with washed packed RBCs to a preoperative hematocrit of at least 35%. Hemoglobin electrophoresis was used to ensure a hemoglobin A level of at least 60%. It should be remembered that 40% of the patient's blood volume is composed of RBCs, which contain only sickle hemoglobin, and these may sickle despite the measures presented earlier. It has been the experience of hematologists that at levels of hemoglobin A of 70% or higher, vaso-occlusive complications usually are abolished.[16]

In our series, patients required an average of 5 days of hospitalization. This was not by design, but of patient necessity based on preoperative and postoperative needs. Five days is clearly beyond present Diagnosis Related Groups (DRG) requirements even with so-called "comorbidity" measures. These patients are in a high-risk group with potentially devastating outcomes, and one should avoid nonoptimal preoperative care or early discharges.

Increased P_{ACO2} during sleep was significant for determining the degree of upper airway obstruction and theoretically the severity of OSAS. This is supported by the fact that increased P_{ACO2} appears to be a more sensitive indicator of hypoventilation than O_2 desaturation. A previous study in normal children with ATH in Sweden demonstrated similar findings regarding decrease in P_{ACO2} and accelerated growth following adenotonsillectomy.[17]

Hypoxia, hypercapnia, and acidosis all have been described as inciting factors in the development of vaso-occlusive crisis, and OSAS may produce all of these physiologic changes. This fact may account for the anecdotal observation that many pain crises appear to come in the middle of the night. We attempted a retrospective review of patient records to determine this association, but inconsistencies in record-keeping and the use of multiple emergency areas precluded any dependable analyses. A prospective analysis is in progress. Whether or not adenotonsillectomy will improve the frequency of vaso-occlusive complications in SCD patients with OSAS remains to be seen.

Following surgery, the patients and their families noticed a subjective improvement in quality of life. This was supported by multiple observations from patients, parents, family, and even teachers.

Growth and development gains in surgery patients are encouraging. These increases were sustained in some patients up to 2 years following adenotonsillectomy. This suggests a marked benefit from surgery in these patients.

Prevalence data suggest that OSAS may be more common in the SCD population than previously known. Comparison to the general population would be intriguing but not valid, as normative data are not available on the incidence or prevalence of OSAS in other populations. Confirmatory data from other centers are encouraged.

We do not suggest the need for adenotonsillectomy in all SCD patients. However, when the appropriate indications from physical examination and sleep studies are present, these patients may benefit from adenotonsillectomy.

The cause of ATH in SCD has not been established. Compensatory hypertrophy of lymphoid tissue may occur in SCD and may increase following "autosplenectomy." This may contribute to the enlarged adenoid and tonsillar tissue found in some SCD patients.

Adenotonsillar hypertrophy may be a response to repeated upper respiratory tract infections because of decreased opsonization of bacterial pathogens, ie, *Pneumococcus*. However, we have not found any data or reference supporting this theory.

Adenotonsillar hypertrophy may occur because of increased hematopoietic needs secondary to the hemolytic anemia. However, hematopoietic centers have not been demonstrated in the adenoids and tonsils of these patients examined by our pathologist, and we can find no reference to support this.

Regardless of the cause, ATH resulting in OSAS occurs frequently in SCD patients. Adenotonsillectomy offers improvement in sleep disturbances and probably improvement in growth velocity of these patients. Surgery may be performed relatively safely in SCD patients with proper attention to preoperative and postoperative details.

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NINDS NOTES*

NINDS ANNOUNCES NEW CAREER DEVELOPMENT AWARD RECIPIENTS

Ten new Clinical Investigator Development Awards (CIDAs) and two Research Career Development Awards (RCDAs) have been made by the National Institute of Neurological Disorders and Stroke. The awards were approved by the National Advisory Neurological Disorders and Stroke Council, which met in October 1988. The awardees and their project titles are as follows:

Clinical Investigator Development Awards

- Denis I. Altman, MB, BCh, Washington University School of Medicine, St Louis, "PET in Cerebrovascular Disorders of Newborn Infants."
- Phillip F. Chance, MD, University of Utah Medical Center, Salt Lake City, "Genetic Analysis of Charcot-Marie-Tooth Disease."
- Marc D. Coltrera, MD, University of Washington, Seattle, "Head and Neck Carcinoma Cell Kinetics."
- Mark R. Gilbert, MD, The Johns Hopkins Hospital, Baltimore, "Neuronal Cytoskeleton in Culture Models of Injury and Repair."
- D. Jeffery Higginbotham, PhD, State University of New York at Buffalo, "Communication Device Effect on Conversational Performance."
- John J. Laterra, MD, PhD, The Kennedy Institute for Handicapped Children, Baltimore, "Adhesion Molecules of Brain Microvessel Cells."
- Gregory P. MacDonald, MD, University of California, San Francisco, "Models of Down Syndrome Based on Genetic Homology."
- Michael I. Nerenberg, MD, Scripps Clinic and Research Foundation, La Jolla, Calif, "Transgenic Models of Viral Neuropathy."
- Steven S. Schreiber, MD, University of Southern California School of Medicine, Los Angeles, "Molecular Biology of Memory."
- John M. Shoffner, MD, Emory University, Atlanta, "Molecular Genetics of Maternally Transmitted Disease."

Research Career Development Awards

- Cynthia J. Forehand, PhD, University of Vermont, Burlington, "Innervation of Target-Identified Sympathetic Neurons."
- Sherry L. Rogers, University of New Mexico, Albuquerque, "Laminin and Fibronectin."

Application deadlines for CIDAs and RCDAs are February 1, June 1, and October 1 of each year. Additional information can be obtained from Edward M. Donohue, Deputy Director, Division of Extramural Activities, NINDS, NIH, Bethesda, MD, 20892; (301) 496-4188.

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*This information is prepared monthly by the Office of Scientific and Health Reports, NINDS, NIH, Bldg 31, Rm 8A-06, Bethesda, MD 20892; (301) 496-5924.

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INTERLEUKIN-2 RELEASE FROM LYMPHOCYTES OF PATIENTS WITH HEAD AND NECK CANCER

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Interleukin-2 (IL-2) plays a central role in cellular immune regulation. In vitro this cytokine is secreted after mitogenic stimulation with phorbolester (PMA), phytohemagglutinin A (PHA), or the monoclonal T cell antibody OKT3. In our experiments the IL-2 concentration was measured in a biologic system using an IL-2–dependent murine cytotoxic T cell line. Control subjects who were age-matched to patients with head and neck cancers did not show an altered IL-2 production in vitro, irrespective of whether they were accustomed to high alcohol consumption or cigarette smoking. Patients with cancer of the larynx did not differ significantly from control subjects. Most prominent was the significant reduction in IL-2 release of patients with cancer of the oropharynx and in patients with positive lymph nodes in the neck. Impaired IL-2 secretion in head and neck cancer patients may therefore be an additional prognostic indicator worthy of further investigation.

KEY WORDS — cancer of oropharynx and larynx, interleukin-2, mitogens.

INTRODUCTION

Malignant tumor cell growth and invasion is controlled by the immune system. Only recently has the importance of cytokines in the regulation of the immune responses been discovered. A major protein with hormonelike characteristics is interleukin-2 (IL-2). Since receptors for IL-2 are found on T cells, B cells, natural killer (NK) cells, and macrophages, it must play a central role in cellular immune regulation.

Interleukin-2 was discovered by Morgan et al[1] in 1976. In the mouse, Lyt-1$^+$ T cells were identified as IL-2 secretors. Interleukin-2 induces in vitro T cell proliferation. This is why it formerly was called T cell growth factor (TCGF). Furthermore, IL-2 activates cytotoxic T cells[2,3] and increases the clonal expansion of NK cells[4-6] and B cells.[7] Interleukin-2 is released in cultures of human lymphocytes after stimulation with mitogens like concanavalin A and phytohemagglutinin A (PHA), or the monoclonal T cell antibody OKT3, whereas the secretion of macrophages with phorbolester (PMA) is independent of macrophages.

In patients with head and neck cancer the existence of an immune deficiency often has been described.[8] In our experiments we were therefore interested in the mitogen-induced IL-2 secretion. In order to analyze the biologic role of IL-2 in head and neck cancer, the objectives of our study were to answer the following questions:

1. Is a difference in IL-2 secretion demonstrable between age-matched controls and cancer patients with low or high intake of alcohol and tobacco?
2. Does a decreased IL-2 secretion exist in patients with head and neck cancer, especially in the oropharynx, in respect to age-matched controls?

These questions are of particular interest, because cancers of the oropharynx are characterized by a very close contact of tumor and lymphoid cells, in contrast to cancer of the rest of the upper aerodigestive tract, as in the larynx, where only a minimal lymphoid cell and vessel composition is present. This lymphoepithelial tissue formation, as found in the whole of Waldeyer's ring, is unique and is found only to a lesser degree in the gastrointestinal tract and lungs.

MATERIALS AND METHODS

Patients with untreated squamous cell carcinomas of the oropharynx and larynx were analyzed (Table 1). Age distribution and stage as classified by the American Joint Committee were associated with a slight tendency to smaller tumor extensions in the larynx group. Since most head and neck cancer patients are accustomed to large quantities of alcohol and cigarette smoking, we used age-matched healthy control subjects with and without high alcohol intake and cigarette abuse (Table 2). High alcohol and cigarette consumption was defined as 100 g ethanol and 20 cigarettes daily based upon subjectively trustworthy statements.[9]

With personal consent from each donor or patient, 20 mL venous blood was drawn in heparinized syringes (1,000 IU heparin-sodium, Braun, Melsungen, Federal Republic of Germany). Mononuclear cells were separated by Ficoll-Isopaque (density = 1,077 g/mL) gradient centrifugation.

From the Department of Otorhinolaryngology–Head and Neck Surgery, Grosshadern Clinic, University of Munich (Wustrow), and the Institute for Immunology and Microbiology, University of Ulm (Kabelitz), Federal Republic of Germany. Supported by grants from the Deutsche Forschungsgemeinschaft DFG Wu 111/2-1, Wu 111/2-2.

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TABLE 1. SQUAMOUS CELL CARCINOMAS

Patient No.	*Diagnosis*	*TNM*	*Stage*	*Age (yr)*	*Sex*	*Alcohol (g/d)*	*Cigarettes (No./d)*
Carcinoma of oropharynx							
1	Base of tongue	T2N2M0	IV	45	M	120	20
2	Base of tongue	T3N1M0	III	37	M	60	30
3	Tonsils	T3N1M0	III	46	F	70	16
4	Tonsils	T2N1M0	III	59	M	100	5
5	Base of tongue	T2N2M0	IV	52	M	80	20
6	Base of tongue	T1N0M0	I	64	F	0	40
7	Tonsils	T4N2M0	IV	52	M	0	20
8	Tonsils	T3N0M0	III	48	M	160	20
9	Base of tongue	T3N0M0	III	47	M	120	20
10	Tonsils	T3N0M0	III	50	M	60	25
11	Base of tongue	T2N0M0	II	37	M	120	50
12	Tonsils	T2N1M0	III	46	M	320	35
13	Base of tongue	T2N1M0	III	75	M	80	20
14	Tonsils	T2N0M0	II	58	M	80	20
15	Base of tongue	T3N1M0	III	39	M	100	20
16	Base of tongue	T3N1M0	III	46	M	100	20
17	Base of tongue	T4N1M0	IV	57	M	80	15
Carcinoma of larynx							
18	Glottic	T3N0M0	III	79	M	40	25
19	Glottic	T2N0M0	II	49	M	120	20
20	Supraglottic	T4N0M0	IV	60	M	100	10
21	Glottic	T1N0M0	I	54	M	100	20
22	Supraglottic	T3N0M0	III	50	M	10	50
23	Glottic	T3N0M0	III	60	M	100	20
24	Glottic	T3N0M0	III	74	M	60	0
25	Supraglottic	T2N0M0	II	44	M	120	40
26	Supraglottic	T1N0M0	I	61	M	0	0
27	Supraglottic	T4N1M0	IV	75	M	20	10
28	Glottic	T3N0M0	III	82	M	150	0
29	Glottic	T3N1M0	III	44	M	120	40
30	Supraglottic	T4N0M0	IV	44	M	40	20
31	Supraglottic	T2N1M0	III	50	M	60	20
32	Glottic	T4N1M0	IV	62	M	60	50
33	Glottic	T2N0M0	II	52	M	90	10
34	Glottic	T2N0M0	II	50	M	100	60
35	Glottic	T2N0M0	II	47	M	80	20
36	Supraglottic	T2N0M0	II	64	M	110	20
37	Glottic	T2N0M0	II	70	M	100	15
38	Supraglottic	T2N0M0	II	49	M	80	25
39	Glottic	T1N0M0	I	46	M	50	10
40	Glottic	T2N0M0	II	64	M	80	5

Viability was determined with trypan blue (0.04%) in Neubauer chambers. As culture medium, RPMI-1640 was used, supplemented with 5% fetal calf serum, 1% penicillin, 1% streptomycin, 1% L-glutamine (Gibco Europe, Karlsruhe, FRG), and 1% 10 m*M* Hepes (Boehringer, Mannheim, FRG). After two washings, the cells (1 x 10^6/mL) were stimulated at 37°C for 24 hours with PHA (Seromed, Berlin), PHA and PMA (Sigma, St Louis), or OKT3 (Ortho Diagnostic Systems, Heidelberg, FRG). The supernatants were frozen at −70°C and stored until analysis for IL-2 concentration.

For the determination of IL-2 we used the IL-2–dependent murine cytotoxic T cell line CTLL.[10] These cells were kept in culture medium RPMI-1640 supplemented with 10% fetal calf serum and 5% supernatants of concanavalin A–stimulated rat spleen cells (2 μg/mL for 24 hours). If no IL-2 is present the CTLL cells die; thus the viability is proportional to the added IL-2 content, since 60 minutes before testing the cells were starting to be cultured in IL-2–free medium. The cell viability was measured by the incorporation of dimethylthiazol-diphenyltetrazolium bromide (MTT; Sigma, St Louis) according to Mosmann[11] and the modification by Heeg et al[12] for a microtiter assay. In triplicates to 100 μL 1.5 x 10^4 CTLL cells/mL 100 μL of IL-2 containing supernatant, medium as negative control, recombinant IL-2 standard (Sandoz, Vienna), or concanavalin A supernatant of tonsil cells was added as a positive control (Fig 1). The color was measured in optical densities by micro-ELISA at 570 nm. From a standard curve with the National Cancer Institute reference the IL-2 concentration was calculated in units/milliliter. The data are

TABLE 2. CONTROL SUBJECTS

Patient No.	*Alcohol (g/d)*	*Cigarettes (No./d)*	*Age (yr)*	*Sex*
1	occ	0	40	F
2	0	0	58	F
3	0	0	55	F
4	occ	0	49	F
5	0	0	52	M
6	0	0	48	F
7	0	0	50	M
8	10	10	52	M
9	5	0	64	M
10	10	1*	61	M
11	50	0	59	M
12	3	0	47	M
13	10	0	64	M
14	0	0	50	M
15	0	0	68	M
16	10	0	69	M
17	occ	0	55	M
18	0	0	59	M
19	20	0	69	M
20	120	20	40	M
21	100	20	54	M
22	100	35	43	M
23	140	20	46	M
24	200	45	47	M
25	140	30	54	M
26	120	20	58	M
27	160	20	58	M
28	160	20	43	M
29	100	30	41	M

occ — occasional.
*Cigar.

presented as median values with the first and third quartiles. Statistical tests were based upon Mann-Whitney rank variance analysis.

RESULTS

After induction with PMA, PHA, or OKT3, the IL-2 secretion increased in vitro with higher stimulation dosages (Fig 2). The mean IL-2 release with PMA was about four times higher than with PHA or OKT3. In our experiments maximal IL-2 secretion was observed after activation with PMA together with PHA. If the optimal stimulation with 25 ng PMA and 5 ng PHA was used, there were no differences in IL-2 release between both control groups with or without high alcohol consumption and cigarette smoking (p> .05) (Fig 3). In laryngeal cancers the values reached similar levels. Lymphocytes of patients with cancer of the tonsil or the base of the tongue, however, produced significantly less IL-2 in vitro than the previously mentioned groups (p< .01).

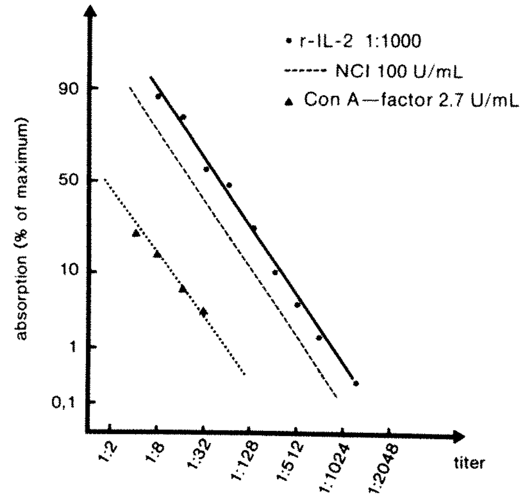

Fig 1. Standard curves of different recombinant interleukin-2 (r-IL-2) probes in microtiter assay. NCI — National Cancer Institute, Con A — concanavalin A.

With PHA as the stimulus, similar patterns were observed (Fig 4). Only the oropharyngeal cancer group secreted, in low concentrations of PHA, less IL-2 than the other groups. Most prominent was the significant reduction in IL-2 production after induction with OKT3 (Fig 5). Again the IL-2 secretion of lymphocytes in oropharyngeal cancers was reduced significantly when compared not only to the controls but also to glottic and supraglottic cancers. Thus lymphocytes from the examined patients with oropharyngeal cancer showed a significant reduction in IL-2 secretion irrespective of which stimulus was used.

DISCUSSION

Interleukin-2 plays a central role in cell-mediated immunity. As one would expect from its first name (TCGF),[13] IL-2 increases the proliferation of thymocytes[14,15] and of IL-2 receptor–bearing T cell clones.[13,16] Moreover, IL-2 induces γ-interferon production[17] and increases NK cell activity[18] and

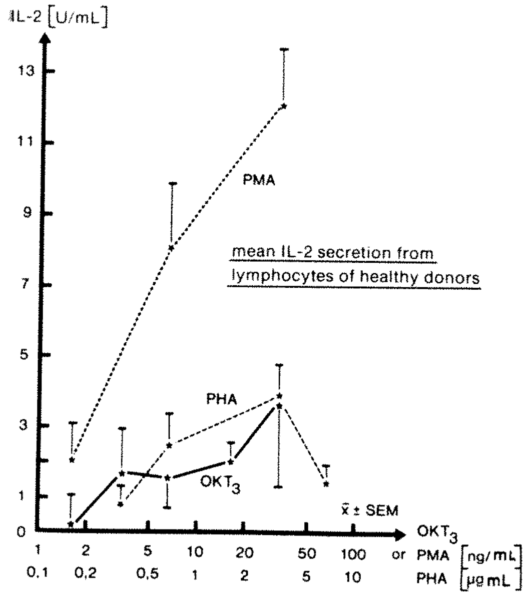

Fig 2. Dose-dependent release of interleukin-2 (IL-2) from lymphocytes in control subjects after stimulation with phorbolester (PMA), phytohemagglutinin A (PHA), or monoclonal T cell antibody OKT3.

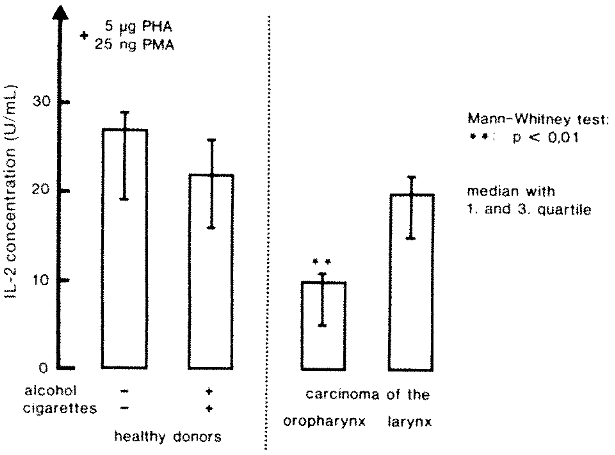

Fig 3. Significant reduction in interleukin-2 (IL-2) secretion from lymphocytes of patients with cancer of oropharynx in comparison to those with laryngeal cancer or age-matched control subjects with or without high alcohol intake or cigarette smoking. PHA — phytohemagglutinin A, PMA — phorbolester.

clonal expansion.[5,6] Furthermore, IL-2 activates the development of antigen-specific cytotoxic T cells[19,20] and regulates dose-dependent suppression of alloreactive cytotoxic T cells in mixed lymphocyte cultures.[21] In addition, the differentiation of antigen-specific T helper cells is improved by IL-2.[22] Interleukin-2 is secreted predominantly by $T4^+$ helper T cells[23]; $T8^+$ cells, however, also have been seen to produce IL-2.[8,24]

After mitogenic stimulation, the secretion of IL-2 and the appearance of an IL-2 receptor at the cell surface is induced.[25,26] Through binding of OKT3, T cells proliferate, express the IL-2 receptor, and secrete IL-2.[27] The antibody OKT3 recognizes 95% of all peripheral blood T cells[28,29] and reacts as mitogen on T cells in concentrations of 1 to 1,000 ng/mL.[29,30] Interleukin-2 production is dose dependent,[31] since high mitogenic concentrations block the differentiation of cytotoxic effector cells against allogeneic targets[32] and inhibit T cell proliferation by soluble antigens. Moreover, the secretion of IL-2 after OKT3 stimulation requires small numbers of macrophages (2% to 5%).[33]

We have been interested in the disturbed IL-2 secretion of patients with head and neck cancer, since this patient group is marked by various imbalances of the immune system. These tumors are immunologically of special interest, because in the oropharynx there exists a close tissue contact between squamous carcinoma cells and the lymphoid cells, allowing direct interactions. In contrast to this location, the larynx consists only of a very small lymphoid cell and vessel composition.

In our experiments we were not able to demonstrate any changes in IL-2 secretion related to the daily alcohol consumption and cigarette smoking habits of healthy donors. Maximal IL-2 release in vitro was observed with simultaneous activation by PMA and PHA. Phorbolester was used for macrophage-independent stimulation,[34] whereas OKT3 is considered to be macrophage dependent.[33] Since the macrophage influence is in the range of 2% to 5%, whereas all our patients had macrophages between 20% and 30%, the differences are not due to alterations in macrophage numbers. The IL-2 secretion was dose dependent for all stimulatory substances. No differences were seen in patients with laryngeal cancer when compared to the previously mentioned groups. In contrast, patients with squamous cell carcinoma of the oropharynx showed a significant reduction in IL-2 production for all stimuli used. The impaired IL-2 release was not due to changes in T cell numbers, since they were low but not significantly different between the groups.

In an overall study of IL-2 production in head and neck cancer patients, Hargett et al[35] and Wanebo et al[36] were not able to demonstrate an impairment of IL-2 secretion using PHA-M as the only

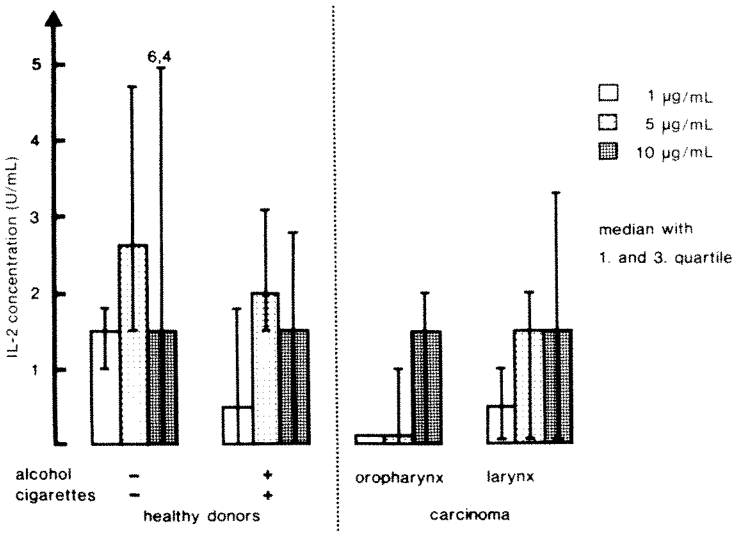

Fig 4. Decreased interleukin-2 (IL-2) release from lymphocytes of patients with cancer of oropharynx after stimulation with phytohemagglutinin, predominantly in low concentrations.

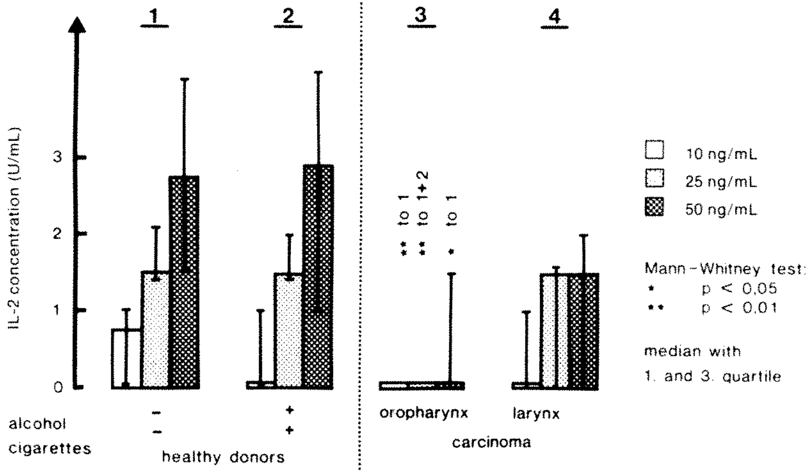

Fig 5. Significantly low, almost absent interleukin-2 (IL-2) secretion from lymphocytes of patients with cancer of oropharynx after stimulation with monoclonal T cell antibody OKT3.

stimulus. However, these authors did not break down the complex group of head and neck cancer patients according to whether the cases were untreated or recurrent, nor according to the site of tumor origin. As the oropharyngeal group consisted of more patients with tumors that involved lymph nodes or in whom lymph node involvement is more likely despite a clinically negative neck, the differences between the oropharynx and larynx groups might well be due to regional lymph node involvement. Although the numbers are very small, a first comparison according to N-stage using a contingency table yielded a χ^2 of 9.2 or $p < .01$. This might indicate a tendency for impaired IL-2 secretion to accompany the presence of positive lymph nodes in the neck and might indicate that IL-2 secretion could be used for biologic staging of head and neck cancer. A thorough analysis, however, will be conducted in future experiments.

In conclusion, the marked immune deficiency often observed in squamous cell carcinomas of the head and neck has been confirmed by the secretion of IL-2, most prominently in cancers of the oropharynx. Whether this is due to the unique close relationship of malignant epithelial tumor cells and lymphoid cells that is present in the oropharynx, or to the amount of regional lymph node involvement, has to be addressed in future experiments. Further research into the nature of the immune defects and a better understanding of the role of the immune system in head and neck cancer are sorely needed to identify those patients in whom adjunctive immunotherapy will be of benefit.

ACKNOWLEDGMENTS — We thank Professor Dr W. Brendel and Professor Dr C. Hammer of the Institute for Surgical Research, University of Munich, for their help.

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G SYNDROME AND ITS OTOLARYNGOLOGIC MANIFESTATIONS

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PORTLAND, OREGON

G syndrome is a familial constellation of congenital anomalies that include a distinctive facies, ocular hypertelorism, prominent occiput and forehead, short lingual frenulum, stridor, hoarse cry, and laryngotracheoesophageal (LTE) clefts, as well as hypospadias and cryptorchidism. Approximately one third of the reported cases have also involved cleft lip and/or palate. Thirty cases of this syndrome have been reported in the pediatric and genetic literature, with variable expression of the syndrome characteristics. The purpose of this article is to report two additional cases of G syndrome with LTE cleft. A technique for repair of LTE cleft via a lateral pharyngeal approach is described. Although G syndrome is rare, otolaryngologists should be aware of its association with LTE cleft and its potential life-threatening problems.

KEY WORDS — congenital anomalies, G syndrome, laryngotracheoesophageal cleft.

INTRODUCTION

G syndrome was first reported in 1969 by Opitz et al,[1] who discussed four brothers of the "G" family with multiple congenital anomalies. Since that time, approximately 30 individuals have been reported with variable expression of the syndrome characteristics.[2]

Affected individuals share a constellation of developmental defects that may be mild or severe in their expression. These include a distinctive facies with ocular hypertelorism, prominent occiput, posteriorly rotated and often abnormal external ears, prominent forehead, male genital abnormalities (such as hypospadias and cryptorchidism), stridor, hoarse cry, short lingual frenulum, and laryngotracheoesophageal (LTE) defects.[1-7] About one third of the reported cases have also involved clefts of the lip and/or palate.[2-4] The great majority of affected patients have been males, although two fatal cases in females have been diagnosed.[7] Mental retardation has been reported in some of the patients, but it is not felt to be associated with the syndrome. The greatest threat to life for these infants is aspiration. We report two additional cases of G syndrome and emphasize the need for the diagnosis and treatment in infants with suggestive signs and symptoms.

LITERATURE REVIEW

The paper published by Opitz et al[1] in 1969 reported four case histories of an apparent congenital syndrome. The four children were brothers, and persistent aspiration occurred in all four. Barium swallow studies in three of the children during infancy demonstrated aspiration, but no specific abnormalities of the esophagus were described. Only one child survived infancy. He required gastrostomy and approximately 2 months of inpatient care with frequent suctioning to prevent aspiration. By 1 year of age he was able to eat without choking. Findings on direct laryngoscopy and esophagoscopy were reported to be normal. In the initial report, his dysphagia was described as functional and was expected to improve with age.

The thoroughness of LTE evaluation was not well documented in the majority of cases of G syndrome reported up to 1978.[1,3,4,8,9] Dysphagia was universally present in all cases reported. Early death occurred in over one third. Surviving patients generally had a history of persistent dysphagia and aspiration in infancy requiring hospitalization with special upright feedings, gavage feedings, or gastrostomy/jejunostomy feedings for prolonged periods.

The first documented case of LTE cleft associated with G syndrome was reported in 1978 by Cordero and Holmes.[5] Diagnosis was delayed in this infant until 3½ months of age, when he was hospitalized with aspiration pneumonia. His previous history included aspiration pneumonia at birth and an episode of congestive heart failure at 1½ months of age. His cleft was diagnosed with endoscopy and was 2 cm in length.

A laryngeal cleft also was reported in 1979 by Greenberg and Schraufnagel.[6] The infant developed bilateral aspiration pneumonia after birth, and a barium swallow study showed a laryngeal cleft. Repair was delayed until he was 5 months of age. Nasojejunostomy feedings failed to provide adequate nutrition and weight gain. He underwent tracheostomy and gastrostomy for persistent dysphagia and intermittent aspiration. His growth and development improved, and repeat endoscopy showed an intact cleft repair. The patient died suddenly at 24 months of age, apparently as a result of aspiration. An autopsy demonstrated a persistent posterior cleft.

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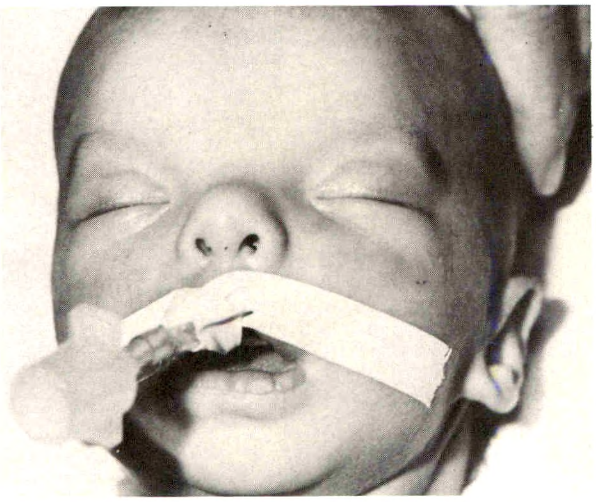

Fig 1. (Case 1) Patient at 1 month of age, intubated prior to endoscopy.

A 1981 report of an infant with G syndrome also mentioned an LTE cleft at postmortem examination.[2] The infant was reported to have incoordination of the swallowing mechanism on esophagram.

Laryngotracheoesophageal cleft is addressed in several articles in the otolaryngologic literature.[10-14] Approximately 60 cases of isolated LTE cleft have been reported in the otolaryngologic literature, and diagnostic evaluation and surgical treatment are thoroughly discussed.

CASE REPORTS

Case 1. This full-term male infant was born on Oct 26, 1980, to a gravida 1, para 0, healthy 19-year-old. The pregnancy was uncomplicated, and the baby was delivered vaginally with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. His birth weight was 3,200 g. The immediate postnatal course was without difficulty, but he developed tachypnea and grunting with feedings during his second day of life. He was transferred to the Oregon Health Sciences University for evaluation. Examination revealed low-set, posteriorly rotated ears, a large but soft anterior fontanel (4.5 x 5.0 cm), a short lingual frenulum, prominent epicanthal folds, and an undescended right testicle. His feeding improved, but he continued to have tachypnea without stridor or cyanosis.

He was discharged after 2 days, but was readmitted at 1 month of age for failure to thrive and feeding difficulties (Fig 1). A barium swallow study demonstrated aspiration (Fig 2). Direct laryngoscopy, esophagoscopy, and bronchoscopy under general anesthesia revealed a laryngeal cleft extending from the arytenoids inferiorly through the cricoid cartilage (Fig 3). He underwent surgical repair of the cleft via a lateral pharyngotomy. Autogenous auricular cartilage was used between the two mucosal flaps to strengthen the repair and to prevent scar tissue retraction below the level of the vocal process. Tracheostomy and gastrostomy were performed.

Ophthalmologic consultation confirmed telecanthus and hypertelorism. Chromosomal studies were normal. Both parents were examined and showed no physical findings of G syndrome. Their histories were negative for feeding or aspiration problems during infancy. The family history of maternal and paternal relatives was also negative. A diagnosis of G syndrome as a result of a new mutation was made.

At 7 months of age the patient had an episode of aspiration pneumonia. His diagnostic workup showed free gastroesophageal reflux, and he underwent a Nissen fundoplication repair. Direct laryngoscopy at that time demonstrated an intact repair of the laryngeal cleft. The patient continued to have feeding problems and aspiration requiring a paraesophageal hernia repair and revision of the Nissen fundoplication.

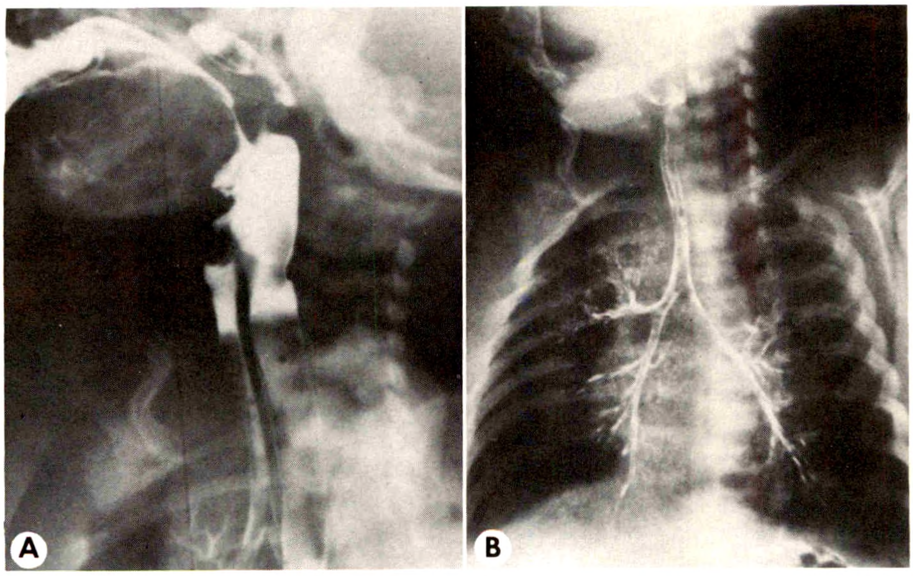

Fig 2. (Case 1) A) Barium swallow study demonstrates aspiration through laryngeal cleft. B) Barium bronchogram secondary to aspiration.

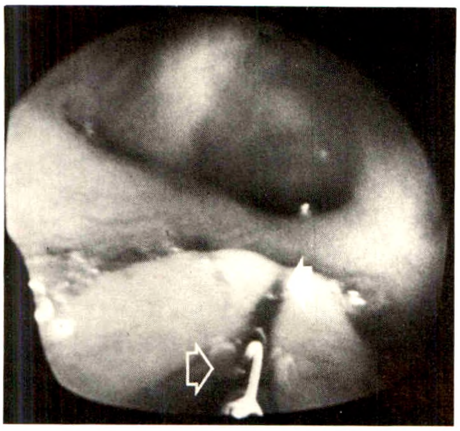

Fig 3. (Case 1) Endoscopic view of cleft larynx. Closed arrow — cleft, open arrow — esophagus.

The patient required continued gastrostomy feedings because he refused to try eating. It was never clear whether this was a psychologic reaction to having always had gastrostomy feedings or a residual of his problems with aspiration and tracheostomy care. Barium swallow study during this time showed some aspiration, but the patient would refuse to swallow and would spit and cough, making interpretation difficult. At about 3½ years of age he started to take some soft sweet foods orally; and over a period of 4 to 6 months he progressed to full oral feedings, allowing the gastrostomy tube to be removed. He had no symptoms of aspiration or recurrent aspiration pneumonia. The tracheostomy tube had been removed successfully when he was 2½ years of age. He was last examined at 4½ years of age and has continued adequate nutrition and development without evidence of aspiration (Fig 4). He has normal true vocal cord motion and voice.

Case 2. This male infant was the product of a 38-week uncomplicated gestation, born by induced vaginal delivery to a gravida 2, para 1 25-year-old. Apgar scores were 1 and 7 at 1 and 5 minutes, respectively.

The infant was noted to have depressed respirations and cyanosis at birth and required mask ventilation. After transfer to the neonatal intensive care unit, intubation was attempted for respiratory distress. The intubation was unsuccessful on multiple attempts because of esophageal intubation. Visualization of the larynx revealed no posterior tracheal wall. Physical examination was significant for bilateral cleft lip and palate with a free-floating premaxilla, large anterior fontanel, low-set, posteriorly rotated ears, hypertelorism, and primary hypospadias. The facial abnormalities are evident in Fig 5A.

Emergency endoscopy was performed, with a diagnosis of LTE cleft extending inferiorly from the level of the true vocal cords for a distance of 2.1 cm to the level of the third tracheal ring (Fig 5B). Tracheal stenosis was evident distal to the cleft. A 2.5-mm bronchoscope was passed through the stenotic segment, but adequate ventilation could not be maintained because the side ports were in the area of the cleft trachea. An intravascular guide wire was passed through the bronchoscope into the right main stem bronchus, and the bronchoscope was removed (Fig 5C). We passed a 2.5-mm Shiley tube over the guide wire and advanced it through the stenotic segment, allowing general anesthesia to be maintained while a tracheostomy was performed. A detailed description of airway management in this patient already has been published.[15] A gastric division was carried out with separate gastrostomy tubes placed into the proximal and distal gastric pouches. Additional medical problems included 1) congestive heart failure with a small ventricular septal defect and 2) a large patent ductus arteriosus (PDA).

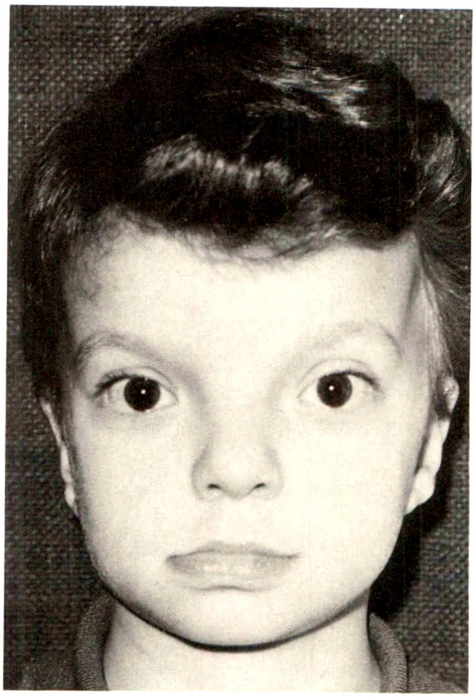

Fig 4. (Case 1) Patient at 4½ years of age, with mild hypertelorism.

The patient had a stormy course with a proximal gastrostomy leak requiring revision when he was 2 weeks of age. A right lip adhesion was attempted and failed when he was 2 months of age. His persistent PDA was ligated when he was 3 months of age to help stabilize his congestive heart failure. His health improved, but he still required continuous ICU care.

When he was 4 months of age his cardiac condition was stable and an LTE cleft repair was accomplished via a lateral pharyngotomy approach. Repeat endoscopy 1 month later confirmed an intact repair with normal true vocal cord motion. A cleft lip adhesion was attempted and failed when he was 5 months of age. His respiratory and cardiac problems stabilized on a multidrug regimen, and he was discharged at 6 months of age with dual gastrosto-

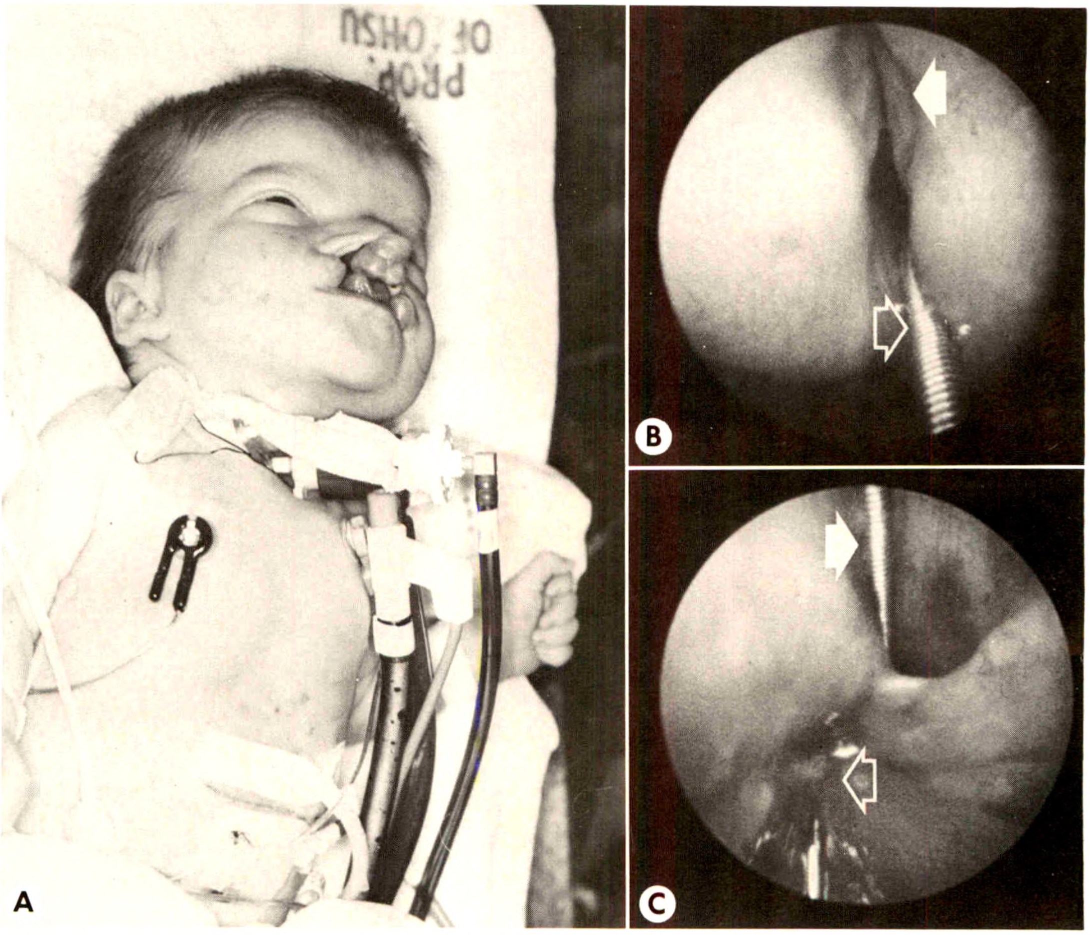

Fig 5. (Case 2) A) After emergent tracheotomy. Note bilateral cleft lip, palate, and depressed midline of forehead. B,C) Endoscopic views. B) Closed arrow — vocal cords, open arrow — guide wire lying in esophagus. C) Closed arrow — intravascular guide wire extending through stenotic area of trachea, open arrow — entrance into esophagus. Between these two arrows, lower edge of cleft is seen.

mies and the tracheostomy tube in place. An episode of viral pneumonia required hospitalization 1 month later. The patient had slow resolution of the respiratory problem and secondary cardiac decompensation. He was discharged on a stable medication regimen for congestive heart failure and reactive airway disease.

The day after discharge he was rushed to the hospital in respiratory distress, and efforts to resuscitate him failed. No autopsy was performed.

Genetic consultation during his first hospitalization included examination of both parents and an older brother. The mother was found to have hypertelorism. The sibling had hypertelorism as well as posteriorly rotated ears. The family history was significant for cleft lip and palate in two maternal uncles. One uncle died shortly after birth from undetermined causes. Chromosomal studies were normal. A diagnosis of G syndrome was made, with mild expression of G syndrome in the older sibling, and the mother as the gene carrier.

DISCUSSION

Inheritance. Evaluation of the cases in the literature indicate that G syndrome is autosomal dominant in inheritance, with partial male limitation.[8] Two cases of female infants with severe manifestations of the syndrome have been reported.[5,7] Several cases of females with mild dysmorphia and dysphagia improving with age also have been reported. Families of affected children frequently have included mothers with hypertelorism and a history of mild respiratory and feeding problems in infancy.[1,3,4,8]

The case 1 patient had an extensive genetics evaluation. No evidence of syndrome characteristics could be found in either parent or any other relative. This child was evaluated by Dr John Opitz (Department of Pediatrics and Medical Genetics, University of Wisconsin Medical School, Madison), who felt that his case represented a new mutation with no evidence of either parent being a carrier. In case 2 there is an autosomal dominant inheritance, giving this family a 50% risk of transmitting the syndrome to their next offspring.

Embryology. The laryngeal cleft defect is believed to develop between the 35th and 45th day of gestation. A sagittal slit forms on the floor of the foregut. This groove deepens into the surrounding

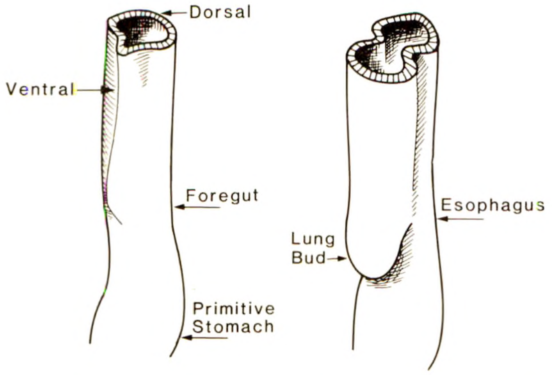

Fig 6. Diagrammatic representation of early development of tracheoesophageal septum. Lateral furrows indent and advance from caudal to rostral.

mesoderm and eventually forms a diverticulum along the ventral surface of the foregut (Fig 6). Lateral furrows indent between the diverticulum and the foregut (ie, esophagus) and advance from caudal to rostral. The resulting tracheoesophageal septum separates the laryngotracheal tube from the esophagus and is believed to be complete to the first tracheal ring by the fifth week of gestation.[10,11]

After the tracheoesophageal septum has reached the level of the future cricoid cartilage, the primitive glottis develops a T-shaped opening due to two lateral swellings that will become the arytenoid cartilages.[16]

The cricoid cartilage develops from bilateral condensing mesodermal centers that fuse ventrally at 6 weeks of gestation and dorsally at 7 weeks. Failure of the dorsal fusion of the cricoid cartilage results in a cleft between the larynx and the esophagus. Some authors believe that the tracheoesophageal septum fails to advance rostrally and results in a lack of fusion of the cricoid cartilage,[5,17] although some case reports have shown clefts of the cricoid cartilage only.[7] Also, one case has been reported with a common tracheoesophageal tube but an intact cricoid cartilage.[11]

Clefts can be divided into three categories, depending on their severity. Type 1 clefts extend into the larynx only. Type 2 clefts extend into the trachea, and type 3 clefts extend the entire length of the tracheoesophageal septum. Clefts that extend into the trachea are fatal without surgical intervention.

Hypertelorism is seen in essentially every case of G syndrome reported in the literature. In the male, hypospadias is seen more than 90% of the time. These two characteristics can be attributed to a basic defect in midline differentiation.[3,4,10] Other midline defects reported in these children have included cleft lip, palate, and uvula; short lingual frenulum; high bifurcation of the trachea; tracheoesophageal fistula; imperforate or displaced anus; duodenal stricture; lung agenesis; and cardiac defects. Dysphagia is seen universally, and in fact, prenatal dysphagia may account for polyhydramnios, which has been reported in some cases of G syndrome, as well as in 30% of isolated cases of LTE clefts.[3,18]

Diagnosis. G syndrome may present as a mild or severe expression of the characteristic morphologic abnormalities. Case 1 illustrates a relatively mild expression, with this child having a delay of diagnosis for 1 month. His diagnosis was made after persistent feeding problems and readmission for failure to thrive. Severely affected individuals as in case 2 have life-threatening respiratory problems at birth, but still these problems may be difficult to diagnose because of equivocal results of diagnostic studies and confusion with CNS or neuromuscular disorders as the cause of aspiration.

Clinical presentation of G syndrome includes hypertelorism and dysphagia in all cases. Aspiration, feeding problems, respiratory distress, cyanosis, hoarse cry, stridor, and excessive secretions are the signs and symptoms of a laryngeal cleft.[2,12,18] The diagnosis is first suggested by the history. Further suspicion should be raised by the physical examination and even the most subtle evidence of midline defects.

The literature on isolated laryngeal clefts cites many cases in which initial evaluation failed to diagnose the abnormality.[10,13,17] Eight of 35 cases reported by Burroughs and Leape[13] had reports of normal esophagrams and endoscopic examinations, and in four of these cases there was negative exploratory surgery. Subsequent diagnosis was made by either repeat endoscopy or autopsy. Five of seven cases of cleft larynx reported by Cohen[10] also were in the category of delayed diagnosis, with prior evaluation including dye studies, endoscopy, and exploratory surgery.

The esophagram may aid in diagnosis, but it can be interpreted wrongly as normal or demonstrate a spillover of barium into the trachea with a tentative diagnosis of a neuromuscular disorder of swallowing. An esophagram seldom will result in a definitive diagnosis.

Endoscopy is required for diagnosis. Even endoscopy can miss clefts if the technique is not meticulous. The edges of the cleft will approximate unless they are stented open. An endotracheal tube will open the interarytenoid area and distal cleft if they exist.[10,12] Esophagoscopy with this "stent" in place will allow visualization of the endotracheal tube through the anterior wall of the esophagus.

Treatment. All survivors of a laryngotracheal cleft have required a tracheostomy and gastrostomy. The LTE cleft should be repaired as soon as the medical condition will allow. We prefer the lateral pharyngotomy approach for repair. It is important that the closure be well above the level of the vocal process and yet not limit lateral rotation of the arytenoids. One problem we have encountered in the past is a partial breakdown of the repair or scar tissue retraction below the level of the vocal process, allowing recurrent aspiration. To prevent this we have used a small piece of conchal cartilage between the tracheal and esophageal suture lines to fill the defect in the posterior cricoid wall.

In those patients with type 2 or 3 LTE clefts, Cotton and Schreiber[12] recommend a double gastrostomy to separate the proximal segment from the distal gastrointestinal tract to prevent reflux, but allow for alimentation. A second problem in type 3 LTE defects is that the tracheostomy tube becomes dislodged above the tracheoesophageal septum into the esophagus because of the short distance between the cleft and the carina.

In case 1 there were continued problems with aspiration and esophageal reflux after the repair. This required further lower esophageal surgery. The patient became psychologically dependent on gastrostomy feedings and was nearly 4 years old before he was able to sustain himself with oral feedings alone. The second patient had a repair shown by examination and esophagram to be intact, but died of presumed cardiac failure at 7 months of age.

Even after repair of the cleft, most children have persistent feeding problems with intermittent aspiration. They may, in fact, have a prominent neuromuscular component contributing to their feeding difficulties. These children require long-term follow-up and reevaluation. Repair of LTE clefts may not be complete, and repeat endoscopy is required to evaluate this. Children with intact repair generally require several months and sometimes years to develop coordinated swallowing.

CONCLUSIONS

G syndrome is certainly rare. Its clinical manifestations range from mild to severe. It is unlikely that all children with G syndrome have an LTE cleft. A posterior laryngeal cleft to the level of the cricoid cartilage or even partially through this cartilage may be compatible with life without any surgical intervention. The majority of the patients diagnosed with G syndrome in the early literature were felt to have a neuromuscular dysfunction of the esophagus creating feeding problems and aspiration. A few of these children were submitted to endoscopy, and those that survived showed definite improvement with age.

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VESTIBULOSPINAL FINDINGS IN TWO SYNDROMES WITH SPONTANEOUS VERTIGO ATTACKS

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The influence of vestibular dysfunction upon the vestibulospinal reflex (VSR) in two common peripheral syndromes was investigated by two types of posturographic examination: "static" posturography, recording and analyzing the postural sway in stance, and "kinetic" posturography, recording the stepping in place test. The influence of the dysfunction was examined outside the attacks, ie, between attacks and not during or immediately after an attack, in patients with Meniere's disease and in others with a sudden vestibular loss syndrome, "neuronitis." However, in both syndromes the influence of the dysfunction was obvious only in some patients; this indicates that central adaptation intervened in the other patients. In this way, central compensation related to the VSR was assessed and compared with compensation for the vestibulo-ocular reflex assessed by the rotation tests. Discordance was shown in a number of cases, ie, a number of cases showed compensation for one reflex, but not for the other. Comparison of the results of both posturographic methods also showed discordance. Normal performance in the walking test could not always be correlated with normal performance in the standing test and vice versa.

KEY WORDS — Meniere's disease, posturography, stepping test, unilateral vestibular hypofunction, vestibular compensation, vestibular neuronitis, vestibulospinal examination.

INTRODUCTION

In peripheral vestibular disease, two typical syndromes are commonly identified: Meniere's disease,[1] and a syndrome with a sudden vestibular loss, called "neuronitis."[2-4] A common feature in both syndromes is that an attack of spontaneous vertigo occurs. However, in Meniere's disease the attacks are recurrent and there is a typical cochlear deficit.[1] In "neuronitis," only one attack occurs and there is no hearing loss. For both types, central disease must be ruled out.[2] Moreover, in both syndromes a permanent dysfunctional state of the vestibular system is supposed to be present: in Meniere's disease the labyrinth is prone to repeated attacks (because of a state of hydrops?) and in neuronitis there is a persisting unilateral loss of vestibular function.

This study examines whether these two dysfunctional states influence the global balance function outside the attacks, ie, between attacks and not during or immediately after an attack, and this especially pertains to the vestibulospinal reflex (VSR). A comparison is made with the effect upon the vestibulo-ocular reflex (VOR).

PATIENTS AND METHODS

PATIENTS

Thirty-nine patients with a typical history of sudden unilateral vestibular loss syndrome (neuronitis) were selected.[2-4] The functional state was confirmed by a unilateral vestibular hypofunction in the caloric testing.[5] The patients were examined 3 to 4 weeks after the initial attack. Central disease was excluded. Seventy-five patients with Meniere's disease were observed. Their typical perceptive hearing loss (unilateral or bilateral) was recorded. Thirty-six of these patients also showed a caloric asymmetry.

METHODS

Each patient underwent a complete otoneurologic examination,[5,6] and the following data were considered for this study.

Caloric Tests. A bithermal technique using 30°C and 44°C water stimulation was applied. The nystagmus obtained was evaluated by measuring the maximal slow phase velocity. Asymmetry was calculated by the formula $R-L/R+L \times 100$ where R equals right and L equals left, and a difference of 30% or more was considered pathologic.

Rotation Tests. The maximal slow phase velocity of the nystagmus, obtained by two types of rotation tests, a sinusoidal stimulation (0.05 Hz, amplitude 80°, 120°, and 180°) and a stop stimulus (90°/s), was measured.[5] The difference between right and left nystagmus was calculated by the same formula mentioned above.

Static Posturography. The technique used was the following. The patient stood on a balance plate for 1 minute. Postural sway was recorded by an x-y writer and was analyzed simultaneously by computer.[7-9] The surface (S) of the area was calculated by computer as well as the total distance covered by the moving point, length (L). Figure 1 shows the scheme of the technique and some examples of the recording. In this study two tests were performed: one with eyes open and one with eyes closed. This furnished four measurements: S and L measured for the recordings with eyes opened and eyes closed.

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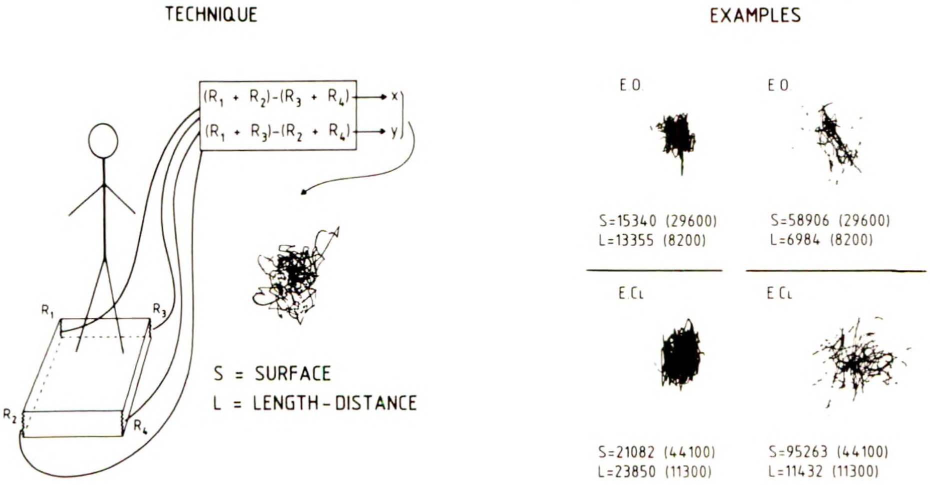

Fig 1. Static posturography. Technique of recording postural sway. Examples of recording by x-y recorder and computing of length (L) in kilograms per centimeter and surface (S) in kilograms per square centimeter. Numbers between parentheses indicate upper confidence limit. EO — recording with eyes open, ECl — recording with eyes closed.

The patient results were compared with results from an age-matched control group. The control group had never experienced vertigo or hearing problems. Posturographic results were considered abnormal when at least one measurement showed a value above $\bar{x}$ + 2SD of those obtained from the control group.

Kinetic Posturography (Stepping Test). The technique was derived from the craniocorpography of Claussen.[10] A light bulb was fixed on each shoulder of the patient. The patient executed the well-known stepping in place for 30 seconds. The movements of the shoulders (oscillations, sway) as well as the displacement of the body were recorded by a photographic method. The shutter of a Polaroid camera was left open during the testing time. The tracing on the photographic plate thus obtained (Fig 2) showed the angle of deviation and/or rotation, as well as the width and regularity of the sway. Only deviation and/or rotation was taken into account for this study, and the limits of the deviation and/or rotation angle were defined by the same method as the limits for static posturography.

RESULTS

In most cases the posturographic recordings showed normal performance (Table 1). Nevertheless a significant number showed abnormal findings, that is, 40% in the static test and 30% in the kinetic test, when considering both examinations in the eyes closed condition. When the kinetic test was compared to the static test in the eyes open condition, a nearly equal percentage showed abnormal results. The difference between the results of the two methods in the eyes closed condition is statistically significant ($p < .02$). The findings were identical for both groups of patients, those with neuronitis and those with Meniere's disease.

Concordant results, ie, normal or abnormal results in both tests for the same case, were observed in the majority of cases, 42 (56%) and 24 (62%), re-

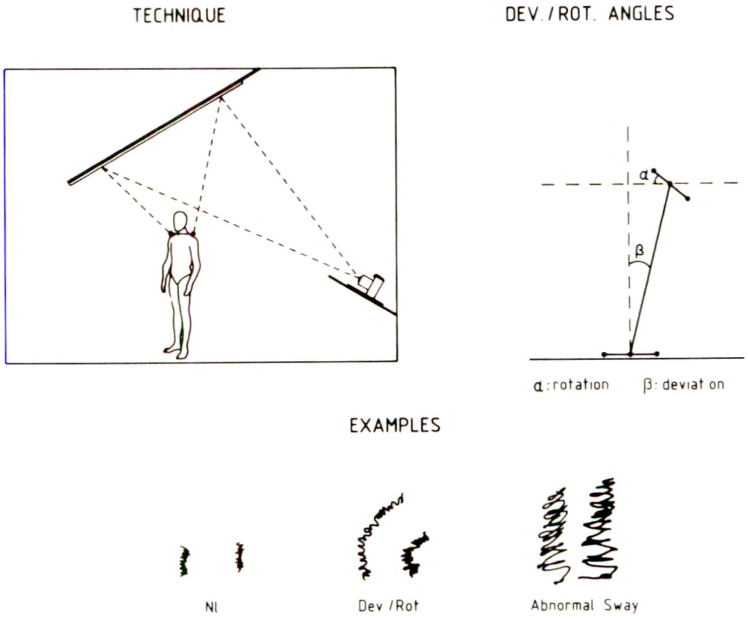

Fig 2. Kinetic posturography. Recording of stepping test by photographic device. Movements of shoulders are recorded by tracing effectuated by light bulbs on film. Shutter of camera is open during testing time of 30 seconds.

TABLE 1. ABNORMAL RESULTS IN POSTUROGRAPHY

	Static Posturography		*Kinetic Posturography*		
	*Eyes Open**	*Eyes Closed**	*Deviation/ Rotation*	*Sway*	*Total*
Neuronitis (n = 39)	10	17 (43.58%)	10	2	12 (30.76%)
Meniere's disease (n = 75)	25	32 (42.66%)	20	1	21 (28.00%)

*At least one measurement (L or S) is positive.

spectively (Table 2). This difference is not statistically significant. On the other hand, in both groups, a significant number of cases, 33 and 15, respectively, showed discordant results, ie, the one test having normal and the other abnormal results.

A comparison of the posturographic data with those of the rotation tests is illustrated in Table 3. It is obvious that in Meniere's disease a smaller number of patients showed abnormal rotation test results ($p < .01$). Moreover, the presence of an asymmetric reaction in the rotation tests was not always accompanied by abnormal posturographic results. Concordance was seen more frequently in the patients with Meniere's disease than in those with neuronitis ($p < .01$).

DISCUSSION

In the otoneurologic approach to the dizzy patient, history-taking will indicate two main types of vertigo: spontaneous and provoked.[6,11] The first type occurs suddenly and unexpectedly regardless of whether the patient is immobile or moving. Provoked vertigo occurs only when a movement (or change of position) is made. Spontaneous vertigo is elicited by a suddenly occurring dysfunction causing a vestibular sensory input that produces a subjective sensation of movement, although the patient is at rest. This type of vertigo disappears when the dysfunction fades away or when a sufficient central compensation is developed. Provoked vertigo is based upon a persisting stable dysfunctional state that has no effect when the patient is at rest. Only certain functional conditions, such as movement, will render the dysfunction manifest because of insufficient central compensation. The vertigo occurs during the movement and lasts only a few seconds.

Among the patients complaining of spontaneous vertigo, two typical categories are common. In both categories the vertigo is typically rotatory and is long-lasting (at least several minutes, hours, or even days). However, in one category, the patient history indicates a single attack; in the other, several recurrent attacks are mentioned. In the first category, the dysfunction involves a sudden unilateral loss of function, which is also called neuronitis[2] or neuritis.[4] Discussion is ongoing as to whether the causative mechanism is a viral infection[4] or a neurovascular incident.[3,12] The syndrome is very common and characteristic. In all such cases cochlear function is normal,[2] and central disease is absent at the time and on further follow-up. The caloric asymmetry indicates the degree of unilateral vestibular loss. In the second group, the attacks are due to a labile functional state of the labyrinth (hydrops?), and in a number of patients, a typical cochlear deficiency is found. In this event, the diagnosis of Meniere's disease can be confirmed.[1]

For this study two types of peripheral vestibular disease were considered: typical Meniere's disease and sudden loss of function called neuronitis. In patients with either type, a persisting dysfunction remained in the absence of actual symptomatic attacks.

We attempted to evaluate how far this dysfunctional state disturbed the global balance function, especially the vestibulospinal function. Peripheral vestibular dysfunction may influence VSR as well as VOR. Influence upon VOR is commonly observed as spontaneous or positional nystagmus and asymmetry in the rotation tests. For both VSR and VOR this disturbing influence is counteracted progressively by the central adaptive mechanisms of compensation, but not for each patient and at each

TABLE 2. CONCORDANCE AND DISCORDANCE BETWEEN RESULTS OF BOTH POSTUROGRAPHIC TESTS

	Meniere's Disease (n = 75)			*Neuronitis (n = 39)*		
	SPG + (n = 35)	*SPG − (n = 40)*	*Total*	*SPG + (n = 19)*	*SPG − (n = 20)*	*Total*
KPG +	11*	9	20	7*	3	10
KPG −	24	31*	55	12	17*	29
Concordant	42			24		
Discordant	33			15		

SPG — static posturography, KPG — kinetic posturography.
*Concordant results.

TABLE 3. CONCORDANCE AND DISCORDANCE BETWEEN RESULTS OF ROTATION TESTS, STATIC POSTUROGRAPHIC TEST, AND KINETIC POSTUROGRAPHIC TEST

	Meniere's Disease (n = 75)			*Neuronitis (n = 39)*		
	RotT + (n = 15)	*RotT − (n = 60)*	*Total*	*RotT + (n = 19)*	*RotT − (n = 20)*	*Total*
SPG +	12*	23	35	6*	13	19
SPG −	3	37*	40	13	7*	20
Concordant	49			13		
Discordant	26			26		
KPG +	4*	6	10	4*	6	10
KPG −	11	54*	65	15	14*	29
Concordant	58			18		
Discordant	17			21		

RotT — rotation tests, SPG — static posturography, KPG — kinetic posturography.
*Concordant results.

TABLE 4. INFLUENCE OF PHASE UPON POSTUROGRAPHIC AND ROTATION TEST RESULTS IN MENIERE'S DISEASE

Phase	*Abnormal Posturography*	*Rotation Test Asymmetry*
1 (n = 37)	13 (35.13%)	8 (21.62%)
2 (n = 25)	11 (44.00%)	6 (24.00%)
3 (n = 13)	11 (84.61%)	1 (7.69%)

moment of the evolution in an equally effective way. This means that at the moment of the examination, one patient is sufficiently compensated and has a normal posturography, whereas another one has insufficient compensation and shows still abnormal posturography. An abnormal posturography means a still disturbed VSR and, consequently, insufficient compensation related to posture.[13] Commonly, this compensation is measured only by examination of the VOR, for which the rotation tests are the most important.[6,14] The effect of the peripheral vestibular disturbance upon the vestibulospinal function can be measured by the posturographic methods described.[7-9]

In stance, a postural sway is necessary for counteracting the force of gravity. Enhanced or disorganized sway is a sign of disturbed stance function. If the VSR is disturbed, its effect upon the postural sway can be recorded and analyzed by the parameters used in the static posturographic test.

While walking, the human body oscillates. In darkness, without visual support, the vestibular and proprioceptive systems can give sufficient orientation to the stepping activity. If, however, in this circumstance the vestibular system furnishes an asymmetric sensory input and if the centers fail to correct it in a sufficient way, a deviation and/or rotation of the stepping direction results. Measuring this aspect of the stepping performance assesses compensation for the VSR related to the kinetic aspect. The width of the sway is rather a measure of central regulation and would indicate central disease.[10] These posturographic data are compared with the results of the rotation tests assessing compensation for the VOR.

In other studies[8,9] it has been shown that the posturographic examination led to a subdivision of the patients examined into two categories: those with values obtained in normal subjects and those with values above the limit of normality. The proportion between both categories approximated 50/50 for the static test. This result was confirmed in the present study (Table 1). However, normal results were observed more frequently in the kinetic test. This held for the two groups of the present study.

The findings indicated that the postural function was different in both categories. They suggested that in the cases with abnormal posturographic results, central compensation for the vestibulospinal aspect was insufficient and could not cope with the disturbed peripheral influence. Comparison of the results obtained by both types of posturographic tests showed that, although in the majority of cases both tests indicated concordant results, for a nonnegligible number of cases the results were discordant. This finding indicated that it could be possible that only one functional aspect — static or kinetic — was disturbed (Table 2). Stepping and standing are two performances obviously organized in different ways so that the disturbing peripheral vestibular input can have an effect on one and not on the other.

In classic examination, only rotation tests are used to evaluate the global balance function, but then only the VOR is considered.[6,14] The present study emphasized that not always must there be concordance between the results of the rotation tests and those of the posturographic tests (Table 3). This was true for both types of posturographic tests. More concordance between rotation tests and posturography was found in the patients with Meniere's disease. Rotation tests gave significantly less abnormal results in the latter cases (Table 4).

The data of this study provided evidence that in a number of patients both persisting peripheral dysfunctions can influence the vestibulospinal function beyond the periods of attack. From this and other studies[8,9] it is evident that posturography adds complementary information, which has mainly functional significance. Posturography alone will not establish the diagnosis of Meniere's disease and cannot supplant the basic classic tests. However, in order for us to better understand the patient's complaints, the data obtained by posturography may be indispensable. In the two syndromes considered in this study, there was still influence upon the VSR outside the attacks in a number of patients. Such an influence can be ruled out by central compensation, as is shown in patients with Meniere's disease and with normal posturographic results.

The functional data provided by posturography are contributory, especially in plans for rehabilitation treatment.[11] When abnormal posturographic results indicate disturbance at the vestibulospinal level, exercises aiming to improve this aspect should be included in the treatment schedule.[15] Moreover, posturography can be of value in monitoring the follow-up of patients.

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MONA LISA SYNDROME: SOLVING THE ENIGMA OF THE GIOCONDA SMILE

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The Mona Lisa smile is presented as a possible example of facial muscle contracture that develops after Bell's palsy when the facial nerve has undergone partial wallerian degeneration and has regenerated. The accompanying synkinesis would explain many of the known facts surrounding the painting and is a classic example of Leonardo da Vinci as the compulsive anatomist who combined art and science.

KEY WORDS — Bell's palsy, contracture, synkinesis.

The art historian Calder[1] noted that the "Gioconda smile" rates with the riddle of the sphinx as an enigma that has teased generations of observers. Ironically, both these mysteries are associated with a pyramid.

In the case of the Mona Lisa smile, the answer lies in the facial nerve, which courses its way through the "aqueduct of Fallopius" deep within the petrous pyramid of the temporal bone. I suggest that the Mona Lisa smile represents a piece of topographic anatomy. Leonardo da Vinci, the compulsive anatomist, painted a classic example of contracture of the facial muscle such as develops after a facial paralysis (Bell's palsy) after the facial nerve has undergone partial wallerian degeneration and has regenerated. The accompanying synkinesis, which could not be captured in a static portrait, would explain many of the known facts surrounding the painting.

The enigmatic expression on the face of the Mona Lisa (La Gioconda) has seduced observers into writing copious speculations. All expressive possibilities are open, and much has been read into the smile: it has been described as beautiful, malicious, wry, and sardonic. Freud[2] pragmatically stated that the smile has a real quality and that it invoked in Leonardo a recollection of his mother's smile. The features are too individual for da Vinci simply to have depicted an ideal type. Freud might have solved the riddle of the smile if he had psychologically analyzed the personality of the subject and not the painter.

The Mona Lisa's smile itself probably inspired the picture. Art critics will resent the suggestion that the smile was a piece of topographic anatomy, but the suggestion can be sustained.[1]

Facial muscles differ from other muscles of the body in that they are not attached to underlying bones but instead control movements of the facial soft tissues by inserting directly into the dermis. The facial skin forms folds, usually at right angles to the direction of the contracting musculature. In childhood, the muscles are not well developed, and a thick layer of subcutaneous fat makes facial expression less apparent than in the adult. Similarly, the skin folds induced by facial muscles are accentuated in the asthenic, are masked in the endomorphic, and increase with each decade of life.[3]

Careful notes concerning the anatomy of the mouth and the lips exist in da Vinci's notebooks written approximately during the time that the *Mona Lisa* was painted. In them, da Vinci gave instructions to would-be anatomists as they dissected a head, describing the spatial relations between the extended lips, the contracted lips, the nostrils, and the eyes in facial movements. Da Vinci's words express his thorough understanding of the unique facial muscle action: "The skin which forms the covering of the muscles that draw always points with its wrinkles to the spot where is the cause of the movement... No muscle uses its power in pushing but always in drawing to itself the parts that are joined to it."[1] The Gioconda smile, probably a sublimation of this anatomic dissection, was "a living face which mutely lent itself to continued scrutiny."[1] Da Vinci's training in anatomy lent accuracy to the transient smile that first attracted him to Mona Lisa.

ARTISTIC FACTS

Unlocking the secret begins with a statement from Vasari's *Lives of the Painters, Sculptors and Architects*[4]: "While Leonardo was drawing her portrait he engaged people to play and sing, and jesters to keep her merry." Another quotation is also pertinent: "He knew the ring of nerves and minute fascicles round the mouth, more sensitive than any musical instrument—while pleasure begs around

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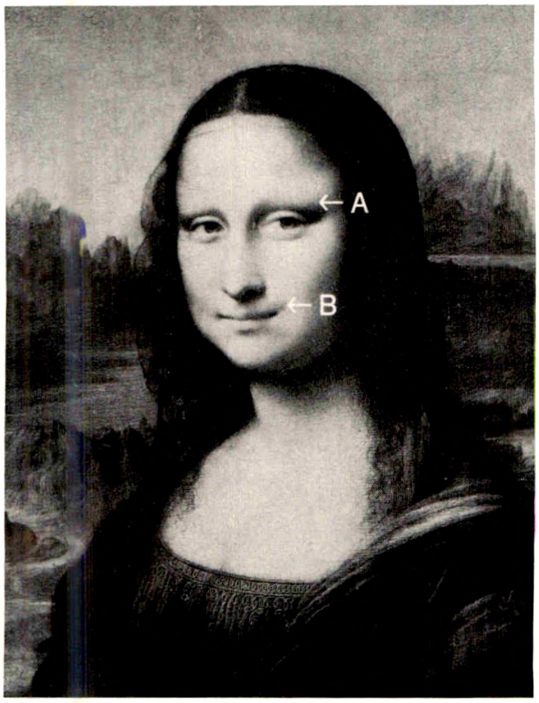

Fig 1. Unretouched photo of *Mona Lisa*. Arrow at A — left interpalpebral distance is less than right interpalpebral distance, arrow at B — left corner of mouth is higher than right corner of mouth (contracture of midface and orbicularis oculi muscles). (Reproduced by permission from Leonardo da Vinci: with an essay by Walter Pater. New York: Phaidon, 1971, plate 27; and by courtesy of the Louvre, Paris.)

the eye to fade sweetly at the corner of the mouth, lifting and shortening the upper lip."[5] Ruskin[6] noted, "Nor are the model's eyes quite the same, nor are the corners of her lips identical. One side curls slightly; the other does not" (Fig 1).

In venturing an opinion from the study of photographs, we can confirm that a similar smile flickered around the mouth of da Vinci's other subjects.[7] His renderings of Saint John the Baptist and Saint Anne have been suggested to have had similar "Mona Lisa" smiles. The original cartoon for *St. Anne With Two Others* (Fig 2), done in charcoal, shows a more symmetric smile than in the final painting (Fig 3). Freud notes, "But although the smile that plays on the lips of the two women is unmistakably the same as that in the picture of Mona Lisa, it has lost its uncanny and mysterious character."[2] Further similarity has been noted between the *Mona Lisa* and the smile of the angel in *Virgin on the Rocks*. The picture, subtitled *Holy Family With Saint Katharine* and now displayed in the Hermitage in Leningrad, also demonstrates the enigmatic smile on the face of Saint Joseph (Fig 4).

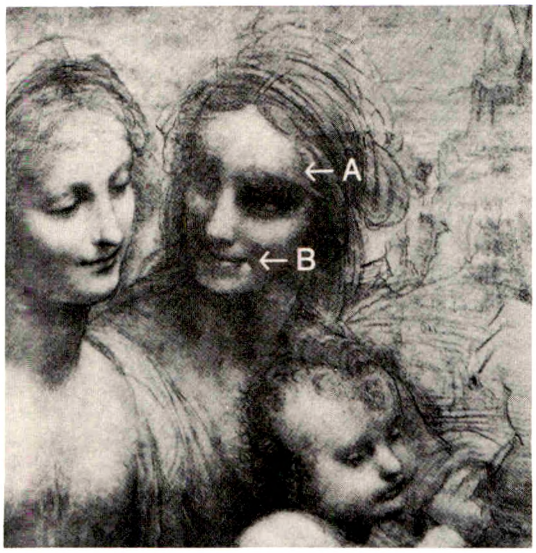

Fig 2. Unretouched detail of cartoon for *St. Anne With Two Others*. Arrow at A — eyebrows (forehead) are not raised, arrow at B — corners of mouth are almost symmetric. (Reproduced by permission from Leonardo da Vinci: with an essay by Walter Pater. New York: Phaidon, 1971, plate 28; and by courtesy of the National Gallery, London.)

The *Mona Lisa* was begun approximately on the subject's 26th birthday (in 1503) and was not completed until her 30th birthday (in 1506). It would therefore seem inappropriate for Keele[8] to suggest that the *Mona Lisa* represents a pregnant woman. Records show that she was the third wife of Francesco del Giocondo, whom she married in 1495. In all probability, Mona Lisa del Giocondo did become pregnant and was the mother of a daughter who died in 1499—before work on the portrait had even begun.

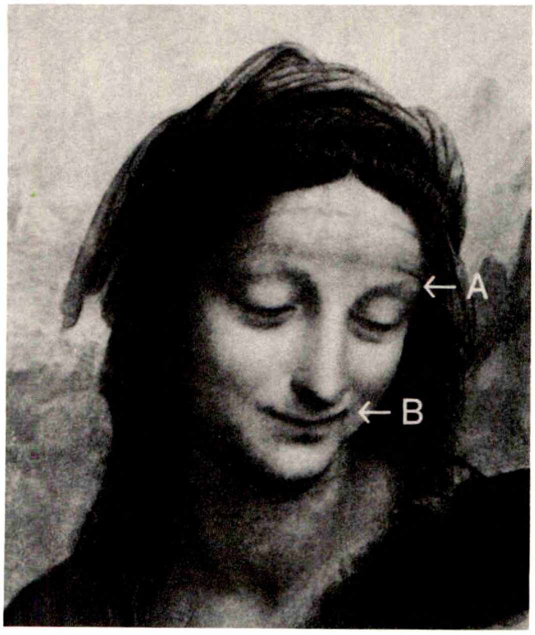

Fig 3. Unretouched detail of final painting of *Madonna and Child With St. Anne*. Arrow at A — eyebrows (forehead) are raised, arrow at B — only left corner of mouth is involuntarily elevated in exaggerated manner (contracture with synkinesis). (Reproduced by permission from Leonardo da Vinci: with an essay by Walter Pater. New York: Phaidon, 1971, plate 30; and by courtesy of the Louvre, Paris.)

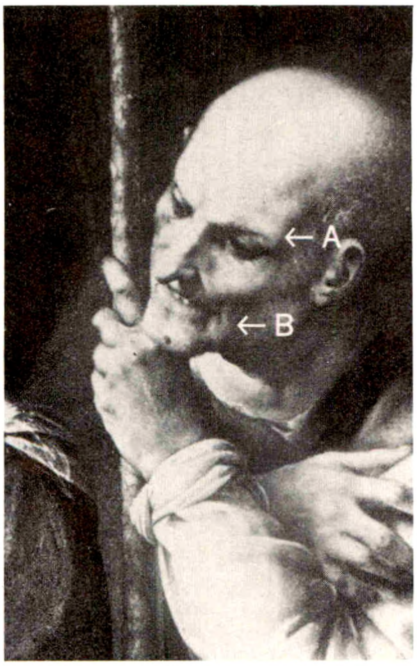

Fig 4. Unretouched detail of Saint Joseph in the painting *Virgin on the Rocks: Holy Family With Saint Katharine* demonstrates exaggerated degree of contracture and synkinesis in left side of midface. Arrow at A — eyebrows (forehead) raised, arrow at B — left corner of mouth grotesquely elevated and showing upper canine tooth. (Reproduced by permission from Rosenberg A. Leonardo da Vinci. Leipzig: Velhagen & Klasing, 1903:129; and by courtesy of the Hermitage Museum, Leningrad.)

MEDICAL FACTS

The pregnancy of Mona Lisa del Giocondo would have increased her risk for Bell's palsy. Bell's palsy is 3.3 times more likely to develop in pregnant women than in nonpregnant women.[9]

In all forms of facial paralysis in which recovery of function is expected (eg, Bell's palsy, Ramsay Hunt syndrome, Guillain-Barré syndrome, trauma), denervation of the facial nerve leads to a predictable outcome. Return of facial function is delayed when any denervation of the facial nerve has taken place. When the nerve does regenerate, usually within 6 to 12 weeks, the sequelae begin, and they become permanent by 16 weeks after onset of the paralysis.

The most common late complications of resolving facial paralysis are contracture and synkinesis (mass motion, associated motion), which occur together. The contracture is represented as a deepening of the nasolabial fold that lifts and shortens the upper lip. This creates "symmetry of the mouth at rest" and has been considered satisfactory recovery from facial paralysis, even though return of facial motion is incomplete. The word "contracture" is actually a misnomer and does not represent fibrous shortening of the affected muscles, but instead a spastic paralysis dependent on the nerve's being intact.

After denervation of the facial nerve, the nerve cell population in the facial nuclei is diminished and is altered physiologically because of retrograde wallerian degeneration. During the reactive phase, which includes the state of chromatolysis, the perineuronal glial cells proliferate. Microglial response may be confined to proliferation; in more severe lesions, however, processes from the microglial cells extend to loosen and then displace the synaptic terminals from the surface membrane of the neuron.[10] The synaptic terminals (boutons) are thought to be inhibitory terminals for cortical control.

Widening of the synaptic gap, and in some cases disappearance of the terminals, isolates the neuron from the surrounding inhibitory influences and thereby leaves the neuron undisturbed to recover and regenerate a new axon. However, when the nerve cell recovers, only a portion of the perineuronal satellites regenerate. With final recovery, there is a reduced or abnormal cell population and reduced cortical inhibition, which causes a true spastic state in the facial muscles.[10]

This postulate is supported by the clinical observation that contracture rarely develops in the frontalis muscles because the superior portion of the facial nucleus (which innervates the forehead) receives inhibitory fibers from both cerebral hemispheres and is not likely to lose inhibitory control. My patients with contracture of the forehead muscles had had bilateral facial paralysis with total loss of electric excitability of both facial nerves.

Degree of contracture and synkinesis are directly related to severity of nerve injury and muscle denervation. Severe contracture and synkinesis develop in patients who have complete denervation. Patients with only partial denervation have contracture and synkinesis directly proportional to degree of denervation.[11] Mona Lisa probably had moderate facial nerve denervation. If a facial nerve is reinjured, severed, or blocked with an anesthetic, the contracture (spastic paralysis) reverts to flaccid paralysis without synkinesis.

Whereas contracture is a static state, synkinesis (mass motion, associated motion) is dynamic and is thought to be caused by misdirection of regenerating nerve fibers[12] or by a "short circuit" in the nerve trunk because of an artificial synapse.[13] With contracture and synkinesis, there develops decreased volitional muscle motion on the affected side as well as involuntary associated muscle action. The movements occur during voluntary and involuntary contraction of the facial muscles. In this way, an involuntary winking movement of the periorbital muscle may accompany voluntary movement of the perioral muscles during speech; conversely, an eyeblink may cause twitching of the corner of the mouth. These movements are recorded electromyographically as "blink bursts" when the recording needle is in the orbicularis oris muscle.[14]

With elevation of the forehead (Fig 3), forced closure of the eye, or exaggerated involuntary smiling, the contracture becomes accentuated and can be grotesque (Fig 4). Children with this sequela learn not to smile.

As early as 1909, Waterman[15] and subsequently a host of other medical authors (including Collier[16]) suggested that patients with contracture and synkinesis should be advised to cultivate "an impassive face." It is thought that attempts at reeducating the facial muscles are likely to accentuate the range of movement and increase the mass movement on the affected side; patients should instead be trained to restrict the range of movement of the affected side and to cultivate a "poker face" so that the normal muscles are trained to restrict movement to match the best that can be obtained on the paralyzed side. In later stages of recovery, excess tone may develop in the affected muscles, leading to overcorrection at rest.

ARTISTIC CONJECTURE

Leonardo da Vinci never really "finished" the Mona Lisa portrait, probably because of the ever-changing relation between eye blink (and its associated motion, upward twitch of the upper lip) and deepening of the nasolabial fold when the forehead moves upward. This would explain why da Vinci used the sfumato technique to make it appear that the figure is being seen through a veil of uncertainty. Da Vinci did not use the sfumato technique in the cartoon line drawing *St. Anne With Two Others* or the painting *Madonna and Child With St. Anne;* therefore, as Freud[2] noted, the smile has lost its enigmatic quality. The expressive purpose of sfumato as da Vinci applied it—in the *Mona Lisa*, at least—was to make the tangible not quite tangible and has more to do with the Mona Lisa's puzzling and disturbing attraction than does the famous smile and curiously removed gaze—neither of whose enigmatic quality can be defined precisely.[17] Further, Mona Lisa del Giocondo had an endomorphic facial structure that would further soften the harsh lines of a contracture.

Leonardo da Vinci, the anatomist, probably was both intrigued and frustrated by the ever-changing position of the nasolabial fold and width of the palpebral fissure. In order to accentuate the anatomic defect, "He engaged people to play and sing, and jesters to keep her merry."[4] Mona Lisa, however, had learned to suppress her smile and to cultivate an impassive face. In his frustration at being unable to capture the dynamic aspect of synkinesis, da Vinci spent many hours painting an elaborate and intricate landscape as a background for the portrait—a practice unheard of in those days.

We have no way of knowing whether Leonardo da Vinci intended his female subject to be the personification of enigma that she has become. If an answer can be discovered in his writings, refutation is as easy to find as support; nevertheless, the analysis offered here is more fact than conjecture. When all the arguments about composition, technique, and detail—and even those about the authenticity of the Louvre version—are exhausted, one point of agreement remains: the Gioconda smile. "If the whole picture dissolved so that, like Lewis Carroll's Cheshire Cat, all that were left was the smile, it would glorify Leonardo."[1]

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OTITIS MEDIA IN THE FIRST YEAR OF LIFE IN TWO ESKIMO COMMUNITIES

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Severe otitis media of early onset is particularly prevalent among Eskimo infants. An investigation of health records of all infants born in 1984 and served by two health facilities was undertaken to assess the relative significance of three particular variables associated with this problem: 1) sex versus age, 2) sex versus severity, and 3) sex versus other health conditions. Ninety-five percent of the children in one locale and 74.4% in the other had at least one episode during the first year of life. While none of the comparisons were statistically significant, infants at the northernmost community were generally more severely affected; females in each community were found to have 1) fewer and less severe episodes of disease, 2) the first episode at a later age, 3) fewer episodes during the year, and 4) fewer episodes of bilateral disease.

KEY WORDS — Eskimo infants, otitis media.

The inordinately high prevalence of otitis media (OM) in the Native American (Indian and Alaskan native) population has been documented by the Indian Health Service (IHS) for the past 25 years. While much had been published concerning the extent of the problem in this group, it has been only relatively recently that a number of variables associated with this prevalence have been identified.

I (unpublished observations) recently reported on three such variables that are of particular significance: age, sex, and racial or genetic factors. In general, OM is found to be highly prevalent in large numbers of children during the first year of life; there is a tendency for more males than females to be affected and to be affected more severely; and prevalence rates are seen to vary extensively with proportion of Indian blood, with certain groups more at risk than others.

In order to study the prevalence and course of disease of early OM in infants from an especially high risk group (Eskimo), medical records on all children born during 1984 in villages served by two Alaskan health facilities of the IHS were analyzed through the first year of life. The two facilities selected are located at Barrow, the northernmost community in the United States, and Bethel, located in west-central Alaska. The Eskimo population served by Barrow during 1984 was 2,843 persons, and by Bethel, 15,140.

Patient records for all children born at all communities served by these facilities were obtained from the IHS Data Processing Service Center, each of which contains diagnoses based on each patient visit. There are marked differences in who the initial service provider may be in each of the two locations, reflecting the relative sizes of medical and ancillary staff. In Barrow, 91.5% of all diagnoses were recorded by general medical officers, followed by medical students (5.75%), community health nurses' aides (1.5%), contract physicians (0.5%), health aides (0.25%), and registered nurses (0.25%). At Bethel, the greatest number of diagnoses were also recorded by general medical officers (37.7%), followed by pediatricians (20.9%), family practitioners (9.7%), nurse practitioners (2.9%), physicians' assistants (1.9%), health aides (1.4%), otolaryngologists (0.97%), pharmacists (0.97%), community health nurses and aides (0.88%), registered nurses (0.48%), cardiologists (0.4%), medical students (0.2%), and orthopedists and internists (0.08%). All episodes of OM were derived from these medical records to provide the data base for this study. Validity of diagnoses is assumed on the basis of 1) ongoing in-service training sessions at each of the facilities conducted by board-certified otolaryngologists from the Alaska Native Medical Center during their specialty clinic visits throughout the year, and 2) substantiation of the earlier diagnoses by a physician on follow-up visits.

All records examined were for visits for illness, with no diagnoses recorded during well-child visits. After examination of these records, a period of 14 days was arbitrarily selected as the cutoff to isolate one episode from another. Separate tabulation of diagnoses of acute (AOM) versus chronic (COM) disease was not practical in that many times the diagnosis was simply stated as OM. Given the ages of the infants, it was assumed that in the absence of the diagnosis "chronic," the episode was of acute disease. The records at Barrow showed no infants whose first diagnosis was of COM, while seven infants at Bethel had this as the first diagnosis. One infant at Barrow had an initial diagnosis of serous OM (SOM), followed later by AOM episodes. Seven infants at Bethel were initially diagnosed with SOM, followed by at least one AOM episode.

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COMPARISON OF SELECTED VARIABLES FOR OTITIS MEDIA IN ESKIMO INFANTS

		Barrow	*Bethel*
Sex versus age at first episode (mo)			
M	$\bar{x}$	5.1	4.6
	Range	0.5 to 12	0.33 to 11
	SD	3.1	2.6
F	$\bar{x}$	5.7	5.4
	Range	1.5 to 9.5	0.5 to 11
	SD	2.6	7.8
		$t = 0.28$*	$t = 0.83$*
Sex versus severity (No. of episodes)			
M	$\bar{x}$	5.0	3.6
	Range	1 to 11	1 to 13
	SD	6.0	3.7
F	$\bar{x}$	4.1	3.1
	Range	1 to 10	1 to 13
	SD	2.9	5.8
		$t = 0.94$*	$t = 0.83$*
Sex versus other health conditions			
M	$\bar{x}$	3.3	3.1
	Range	0 to 6	0 to 9
	SD	1.4	5.0
F	$\bar{x}$	3.4	2.7
	Range	1 to 6	0 to 8
	SD	1.4	5.8
		Not tested	$t = 0.10$*

*Not statistically significant.

Of the infants born in Barrow, 95% (56 of 59) had had at least one episode of OM during the first year of life. Of this number, 37 were male and 19 female. Over half of the infants (56.4%) had three or fewer episodes, with the range being from one to 11. Age-specific attack rate analysis by 3-month periods shows 22 infants (40%) with the first episode occurring between 0 and 3 months, 17 between 4 and 6 months (31%), 11 between 7 and 9 months (1.8%), and five between 10 and 12 months (9%). In the first 6 months of life, 71% of the infants had had at least one OM episode. At least one episode was described as bilateral in 24.3% of the males and 16.6% of the females.

These infants also incurred an average of 3.3 other health problems during the year: bronchitis or bronchiolitis, 23; pneumonia or pneumonitis, 15; conjunctivitis, 12; diarrhea, nine; gastroenteritis, six; viral exanthem, six; anemia, five; hyperbilirubinemia, four; thrush, four; common cold, four; and chickenpox, four. The three infants with no histories of OM incurred an average of 2.6 other conditions during the year.

Of the infants born in Bethel, a much lower rate of OM was reported, with 74% of 285 infants (114 male, 98 female) experiencing at least one episode of OM during the first year of life. A much higher percentage (67.5%) was reported with only three or fewer episodes (50 with one, 58 with two, and 35 with three). The range of episodes was one to 13. The age-specific attack rate was very similar to that obtained for Barrow, with 36% of first episodes occurring between 0 and 3 months, 36% between 4 and 6 months, 23% between 7 and 9 months, and 4% between 10 and 12 months. By the age of 6 months, 73% of the infants had experienced their first episode of OM. At least one bilateral episode was reported for 23.6% of the males and 16.3% of the females.

Other conditions and their frequency were upper respiratory infections, 93; pneumonia or pneumonitis, 50; anemia, 42; bronchitis or bronchiolitis, 35; thrush, 33; conjunctivitis, 29; heart murmur, 28; gastroenteritis, 26; diarrhea, 24; hyperbilirubinemia, 17; pharyngitis, 13; viral stomatitis, ten; viral syndrome, seven; and viral exanthem, six. The children with histories of OM incurred an average of 2.8 other conditions; children with no histories of OM averaged 1.1. By sex, there was an average of 0.97 other conditions for the 31 non-OM males and 1.2 for the 42 non-OM females.

Three comparative conditions for each locale were analyzed: sex versus age, sex versus severity (defined by number of episodes), and sex versus other conditions. These results are summarized in the Table.

While none of the comparisons cited in the Table approached statistical significance, there are still a number of apparent trends that may be of clinical significance.

While the OM rates in infants in both communities are exceedingly high, the disease appears to have been more severe in Barrow in the percentage of infants affected, 95% versus 74.4%. Professionals working in Barrow and Bethel have speculated that the difference between the two locales may be attributed to 1) the ongoing presence of an aggressive OM control program in Bethel and surrounding villages, an area that has traditionally had an exceptionally high prevalence of OM, and 2) the possibility that records on a small number of infants in villages away from Bethel may not have been included in the IHS data base. Geographic location and weather are not felt to be significant in these disease-rate variances.

A comparison of age at first episode by community favors Barrow slightly in both mean age and age range. Measurement of severity by numbers of episodes clearly favors the Bethel infants of both sexes. Severity measured by the numbers of other health problems encountered is nearly equal between the groups.

Disease severity by sex in both communities favors females in nearly all comparisons. Infant girls had their first episode at a later age, had fewer episodes, and were near-equivalent in numbers of other health conditions. Presumed severity of disease based upon one or more episodes of bilateral involvement again favored the female infants in both communities. The homogeneity of the populations on racial and genetic factors obviated the need for comparisons on those variables.

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ACTOMYOSIN ADENOSINE TRIPHOSPHATASE ACTIVITIES OF THE CAT INFRAHYOID MUSCLES

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By use of actomyosin ATPase histochemistry, it was found that there were large differences among the three cat infrahyoid muscles (sternohyoid, sternothyroid, and thyrohyoid) with respect to their percentages of different muscle fiber types. It has been established that the individual activity patterns of the component motor units in each muscle drive the biochemical and physiologic differentiation of the muscle fibers associated with each motor unit. Therefore, the data obtained in the present investigation provide an indication of the characteristics of long-term use of each of the various types of motor units, as well as the associated differences in the physiologic capacities of the different motor unit types composing each of these infrahyoid muscles.

KEY WORDS — actomyosin, adenosine triphosphatase, infrahyoid muscles, muscle fiber types, sternohyoid muscle, sternothyroid muscle, thyrohyoid muscle.

INTRODUCTION

In spite of the fact that the infrahyoid muscles (the sternohyoid [SH] muscle, the sternothyroid [ST] muscle, and the thyrohyoid [TH] muscle) play important roles in such activities as respiration, deglutition, phonation, and singing,[1-14] very little is known about their histochemical characteristics. Since there is considerable evidence indicating that both the physiologic characteristics of the motor unit and the enzyme profiles of the included muscle fibers reflect the pattern of activity of the motor unit,[15,16] data concerning the enzyme profiles of the infrahyoid muscles are essential to an understanding of the biochemical basis for physiologic differences in the motor units in these muscles.

Using a glycogen depletion technique, Burke et al[17,18] directly examined the relationship between the histochemical and the physiologic characteristics of single motor units in the cat. They found that type 1 fibers had the slowest contraction times and were the most resistant to fatigue. Type 2B fibers had the fastest contraction times and were the least resistant to fatigue. Finally, type 2A fibers were reported to have contraction times nearly as rapid as those of the type 2B fibers and to be nearly as resistant to fatigue as type 1 fibers. Subsequent studies have confirmed these relationships between the histochemically defined muscle fiber type and the physiologic characteristics of the respective motor unit.[19,20] In the present study, actomyosin adenosine triphosphatase (ATPase) histochemical techniques have been used to compare the compositions of the cat infrahyoid muscles by muscle fiber type as an indication of the functional differences between these muscles.

MATERIALS AND METHODS

Five female cats ranging in weight from 2.5 to 4 kg were used for this study. They were deeply anesthetized with intravenous injection of sodium pentobarbital (20 mg/kg). An incision was made on the ventral surface of the neck, and samples of each of the right infrahyoid muscles (SH, ST, and TH) were taken at midlength. Each muscle was immediately frozen by rapid immersion in *n*-methylbutane cooled to its freezing point with liquid nitrogen. They were then stored in a liquid nitrogen refrigerator in order to stabilize enzyme activities until they were processed. Transverse sections (10 μm thick) of the muscles were cut in a cryostat, mounted on coverslips, and allowed to dry at room temperature.

Sections then were incubated for alkali-stable (at pH 9.0) and acid-stable (at pH 4.6 and 4.3) actomyosin ATPase according to the method of Guth and Samaha.[21] The muscle fibers types were classified in each of the muscles according to the method of Brooke and Kaiser.[22,23] In this system, muscle fibers are classified into one of four categories (type 1, 2A, 2B, or 2C) on the basis of the characteristics of their myosin ATPase. An initial classification of the fibers into two groups (system of Engel[24]) is made on the basis of their ATPase reaction product density in sections that are preincubated in a solution having a pH in the alkaline range. (We used pH 9.0.) The fibers that have relatively little staining after this alkaline preincubation are categorized as type 1 fibers, and those that stain darkly under these conditions are classified as type 2 fibers. The type 2 fibers are then classified into subgroups on the basis of the extent of ATPase inactivation present when serial sections are preincu-

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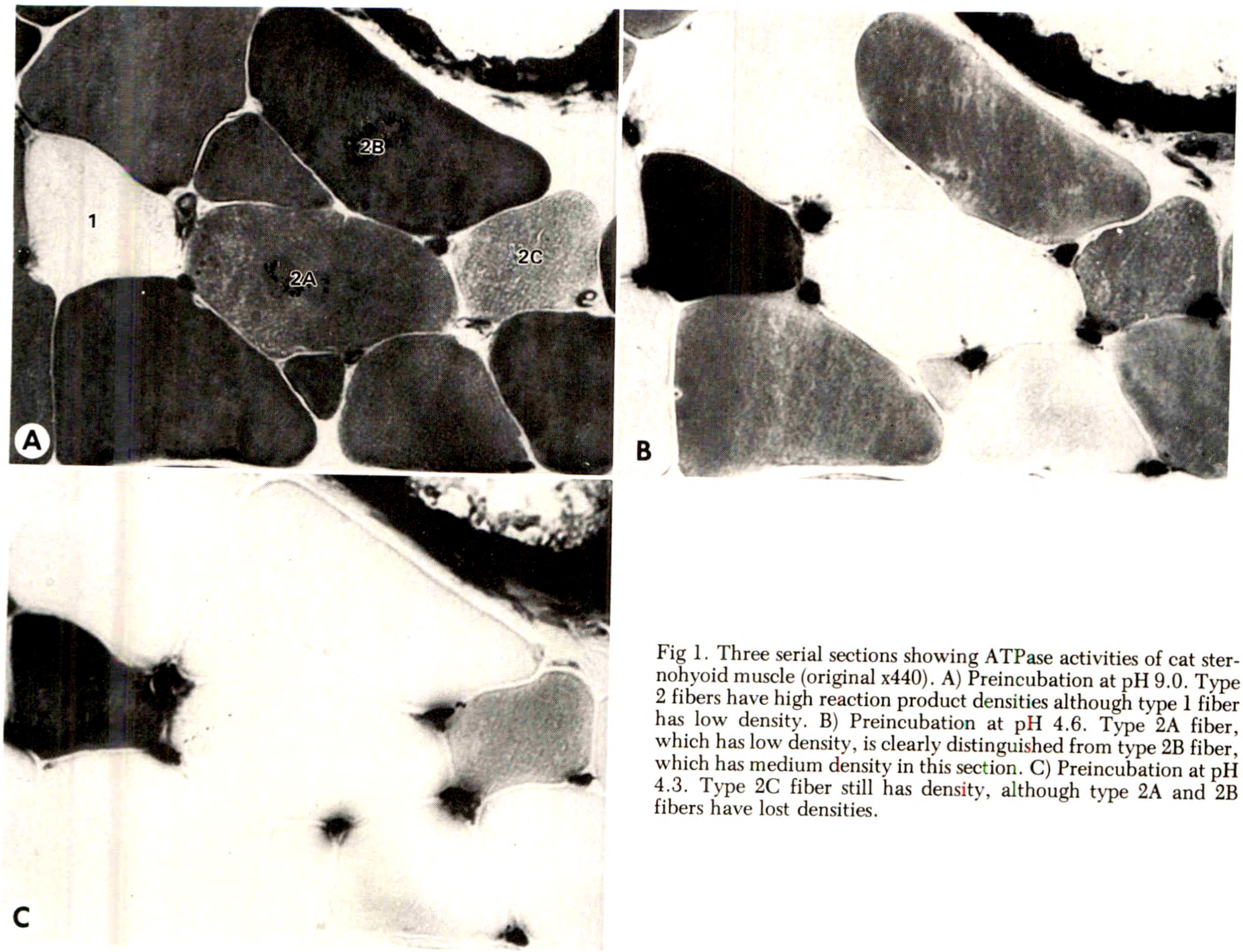

Fig 1. Three serial sections showing ATPase activities of cat sternohyoid muscle (original x440). A) Preincubation at pH 9.0. Type 2 fibers have high reaction product densities although type 1 fiber has low density. B) Preincubation at pH 4.6. Type 2A fiber, which has low density, is clearly distinguished from type 2B fiber, which has medium density in this section. C) Preincubation at pH 4.3. Type 2C fiber still has density, although type 2A and 2B fibers have lost densities.

bated at pH 4.6 or 4.3. Type 2 fibers that have marked inactivation of ATPase activity at pH 4.6 are classified as 2A fibers. Those that retain substantial activity at pH 4.6, but show complete inactivation at pH 4.3, are categorized as type 2B fibers. Type 2C fibers are distinguished from 2A or 2B fibers by their retention of substantial ATPase activity after preincubation at pH 4.3. Since sections stained with preincubation at pH 4.6 were used to count the fiber composition and type 2C fibers have medium reaction densities like those of type 2B fibers in these sections as stated above, type 2C fibers were included in the type 2B group. However, type 2C fibers were very rare or absent in the cat infrahyoid muscles and could therefore be ignored in determining the overall percentages in these muscles. Since there have been reports indicating that in some muscles there is a higher percentage of type 1 fibers in deeper parts of the muscle than in more peripheral regions,[25,26] we examined this possibility by taking samples from two fascicles from the central part of the muscle and also from two fascicles near the surface of the muscle. Since there were no differences in fiber type composition between these two sites, the data were pooled to represent the overall fiber type composition of each muscle. Although it is conceivable that differences in the fiber type composition occur along the length of these very long bandlike muscles, this seems very unlikely since there is, to our knowledge, no evidence in the literature of a fiber type gradient along the length of any long muscle having a simple architecture comparable to that of the infrahyoid muscles. Therefore, a three-dimensional reconstruction of the fiber type architecture was not considered to be within the scope of this investigation, and instead comparisons were made using samples taken at the midlength of each muscle.

We determined the percentage composition of the different muscle fiber types by counting the number of each fiber type and calculating the percentage of the total. The distinction in staining density among the fiber types was clear, and its reliability was confirmed during acid reversal procedures. These techniques have been used widely in many research studies and also are used routinely for clinical applications in connection with muscle biopsies.[17-20,27]

Unless otherwise indicated, an analysis of variance[28] was used to test for the significance of differences among the infrahyoid muscles in the muscle fiber type percentage compositions with a probability value of $< .05$ as a criterion of significance. In

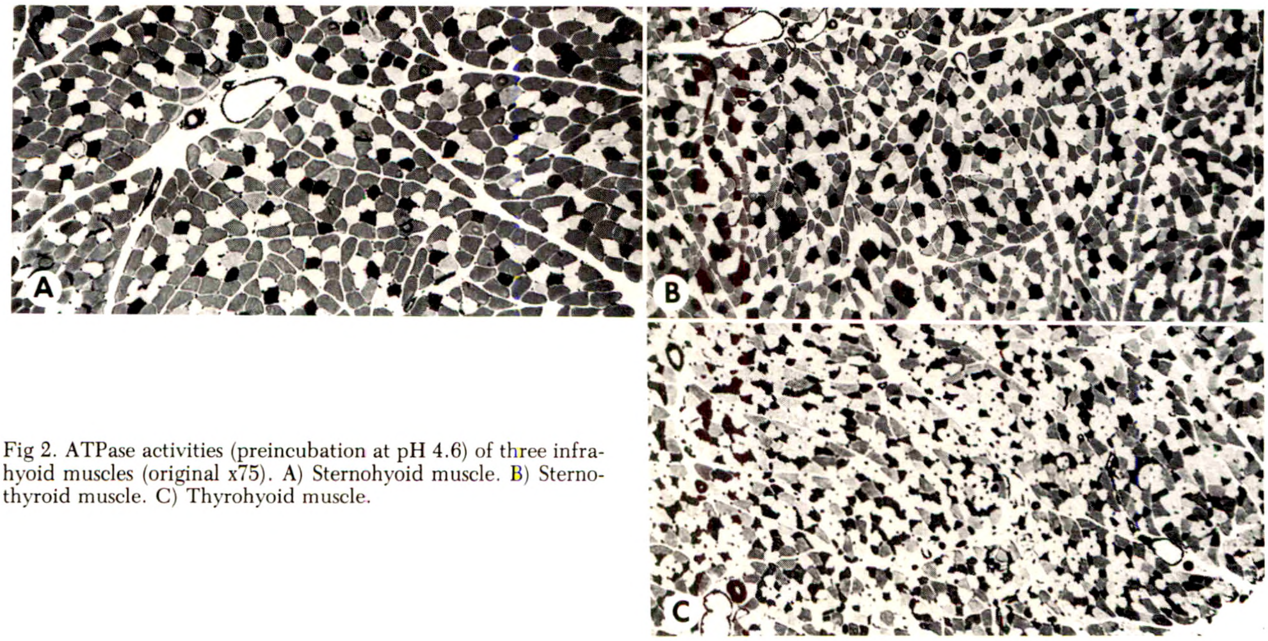

Fig 2. ATPase activities (preincubation at pH 4.6) of three infrahyoid muscles (original x75). A) Sternohyoid muscle. B) Sternothyroid muscle. C) Thyrohyoid muscle.

all analyses the assumption of the homogeneity of variance was tested at the same probability level by use of a robust alternative formulation of Levene's test statistic.[28-30] When it could not be assumed that the variances were equal, the analysis of variance was carried out by use of the Welch statistic, which does not assume the equality of variances in each group.[28,31] The Scheffé test was used for all multiple comparison analyses at the same probability level.[28,32]

RESULTS

With the pH 9.0 incubation (Fig 1A), two muscle fiber types were consistently detected in the three infrahyoid muscles. These fiber types included muscle fibers with low reaction product densities (type 1) and muscle fibers with high reaction product densities (type 2). These densities reversed in the acid preincubations. With the pH 4.6 preincubation (Fig 1B), the densities of type 1 fibers reversed into high densities, and those of type 2 fibers into low densities (type 2A) and medium densities (type 2B). With the pH 4.3 preincubation (Fig 1C), most of the type 2 fibers were essentially unstained, although type 1 fibers still had substantial reaction product densities. However, a few of the type 2 fibers had densities that were lower than those of the type 1 fibers (type 2C). These type 2C fibers were very rare in the cat infrahyoid muscles and not always found in all muscles. Therefore, sections stained for actomyosin ATPase preincubated at pH 4.6 were used to make counts for the determination of the overall muscle fiber type composition in this investigation.

The fiber compositions in the three infrahyoid muscles were very different (Fig 2). The percentage compositions of the three fiber types (types 1, 2A, and 2B) in each of these infrahyoid muscles in each experimental animal are shown in the Table. An analysis of variance indicated significant differences among the infrahyoid muscles in the percentage compositions of type 1 fibers, but the value obtained ($p = .049$) for Levene's test for the assumption of homogeneity of variance indicated that a homogeneity of variance could not be assumed. (The criterion used for nonhomogeneous variances was $p < .05$.) For this reason, an alternative test based on the Welch statistic, which does not assume the equality of variances, was used.[28,31] This test also indicated significant differences in the percentages of type 1 fibers among the infrahyoid muscles. A specific multiple comparison test indicated that these differences were significant among all of the infrahyoid muscles. The differences among the infrahyoid muscles in the percentage compositions of type 2A fibers were also signifi-

PERCENTAGE COMPOSITION OF EACH FIBER TYPE IN THREE INFRAHYOID MUSCLES IN EACH EXPERIMENTAL ANIMAL

	Sternohyoid Muscle			*Sternothyroid Muscle*			*Thyrohyoid Muscle*		
Animal	*1*	*2A*	*2B*	*1*	*2A*	*2B*	*1*	*2A*	*2B*
1	16.1	35.5	48.4	26.8	53.7	19.5	40.5	45.5	14.0
2	9.5	33.9	56.6	22.1	53.3	24.6	46.8	36.7	16.5
3	14.7	29.1	56.2	25.6	49.8	24.6	35.1	40.7	24.2
4	14.9	31.7	53.4	27.5	47.2	25.3	32.5	42.7	24.8
5	13.0	32.7	54.3	22.8	46.8	30.4	33.9	48.8	17.3

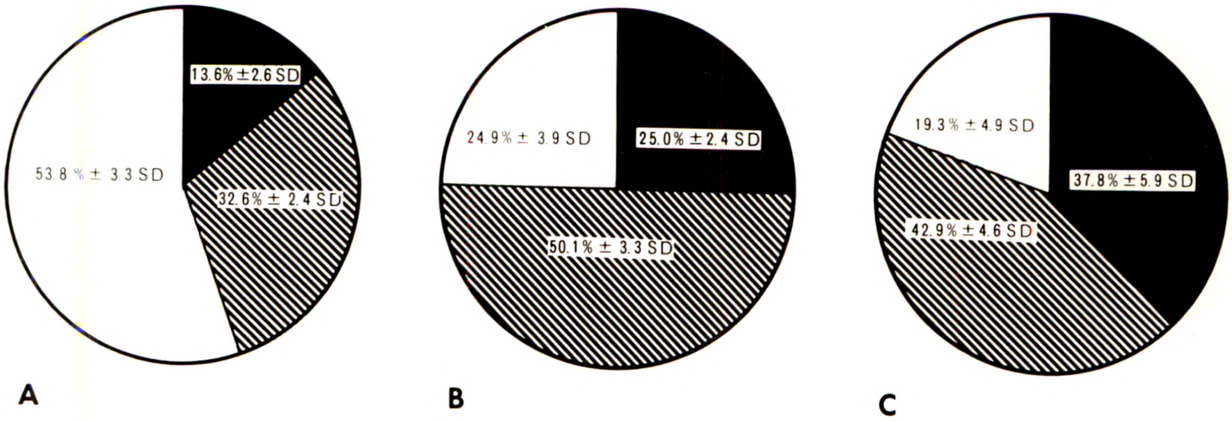

Fig 3. Percentage composition of three fiber types in each infrahyoid muscle (n = 5). A) Sternohyoid muscle. B) Sternothyroid muscle. C) Thyrohyoid muscle. Black — type 1, shaded — type 2A, white — type 2B.

cant, with a multiple comparison test indicating again that these differences were significant for all possible comparisons among the infrahyoid muscles. Significant differences in the percentages of type 2B fibers were also indicated among the infrahyoid muscles. A multiple comparison test indicated that these differences in the percentage of type 2B fibers were significant between the SH and the ST muscles, and between the SH and the TH muscles, but not between the TH and the ST muscles. The percentage composition of the three fiber types in each muscle is shown collectively in Fig 3.

DISCUSSION

The present results for acid reversal correspond well with the muscle fiber type categories established in earlier studies on the limb muscles.[22,23] The fiber types detected in the infrahyoid muscles included all of the fiber types generally reported in the limb muscles.[22,23]

The present study has shown that there are great differences in the percentage composition of type 1, 2A, and 2B muscle fibers among the three infrahyoid muscles examined. The SH muscle had a large number of type 2B fibers, a small number of type 1 fibers, and a small number of type 2A fibers. The fiber composition in the ST muscle fell between those of the SH and the TH muscles.

Since the cat is one of the most widely used animal models in studies of muscle physiology, the results of this investigation may be useful in relation to studies of infrahyoid muscle function that require the use of an animal model. As indicated in the Introduction, glycogen depletion techniques have been used to establish the relationships between histochemically characterized muscle fiber types and the corresponding physiologic characteristics of single motor units in the cat as well as in human muscles.[17-19] According to such an interpretation, the SH muscle would have the highest percentage composition of those fiber types that have the fastest contraction times and the least fatigue resistance. On the other hand, the TH muscle would have the greatest percentage composition of fibers types that have the slowest contraction times and the greatest resistance to fatigue. The ST muscle would be between the SH and TH muscles with respect to these characteristics. A comparison of the results obtained for the cat infrahyoid muscles to those for the human infrahyoid muscles is difficult, since to our knowledge histochemical data are available only for the human ST muscle.[33] That study indicated that the human ST muscle was composed of approximately 70% type 1 fibers and 30% type 2 fibers, compared to corresponding values of 25% type 1 fibers and 75% type 2 fibers in the ST muscle obtained in our present study in the cat. However, before a comprehensive comparison can be made between the muscle fiber compositions of the human and cat infrahyoid muscles, it will be necessary to have more complete data on the human infrahyoid muscles.

In addition to the correlation that exists between the muscle fiber type and its physiologic characteristics, there is also a relationship between the fiber type and the position of the respective motor unit in the recruitment sequence.[34,35] Many studies have indicated that the biochemical and ultrastructural differentiation of a muscle fiber into a specific fiber type is driven primarily by the activity pattern of the associated motor neuron,[15,16,36] which is in turn a function of the position of that motor neuron in the recruitment sequence.[37,38] The percentage composition of the various muscle fiber types that compose each muscle therefore provides an indication of the overall long-term use of the various motor unit types in each muscle. In most systems that have been investigated, it has been found that the sequence in which various motor units are recruited is essentially inflexible and not task-dependent,[37,39,40] so that those units that are recruited early in the sequence are activated very frequently and those recruited last in the sequence are activated only when the muscle generates its maximum tension. Type 1 fibers are recruited earliest in the sequence, and they are well suited to this physiologic role as a result of their great fatigue resistance.[17-19] The recruitment of type 1 fibers is followed by the recruitment of type 2A fibers, with type 2B fibers occupying the infrequently recruited end of the sequence.[19,38] Therefore, our data indicating differences in the muscle fiber type percentage compositions of the infrahyoid muscles probably reflect differences in the ways that each of these muscles are used.

The TH muscle has the largest type 1 component and also the largest type 2A component. This would suggest that much of the total capacity for tension generation in this muscle is very frequently used, since both of these fiber types are associated with frequent recruitment.[19] The relatively high percentage of fatigue-resistant fibers in the TH muscle is probably related to the reported presence in this muscle of an essentially tonic electromyographic (EMG) discharge pattern, which is inhibited only during midinspiratory and late expiratory phases.[4] Murakami and Kirchner[4] have suggested that the function of the tonic activity component in this muscle is to maintain a constant distance between the hyoid and the thyroid cartilages during normal quiet respiration and that inhibition of the tonic activity in this muscle during hyperpnea or dyspnea results in a descent of the thyroid cartilage, an increase in the TH distance, and an increase in the width of the glottic aperture.

The percentage composition of type 2B fibers in the TH muscle, on the other hand, is the lowest of any in the muscles examined; this finding would indicate a relatively small, fatigable reserve component. The recruitment of the 2B fiber component in the TH muscle presumably is related to some more occasional function that necessitates a brief increase in the tension generated by this muscle. This type of activity might be expected during swallowing, which in humans occurs at a frequency of 300 times per hour during eating and approximately ten times per hour during fasting.[41,42] Studies with cats, dogs, and monkeys have shown that the TH muscle shows increased EMG activity during swallowing.[9] It also has been demonstrated that in dogs the TH muscle plays an important role in the elevation of the larynx during swallowing and that this muscle, with the cooperation of the suprahyoid muscles, is capable of producing closure of the larynx even when the contribution of the intrinsic laryngeal muscles innervated by the recurrent laryngeal nerve is eliminated.[7] It is also likely that intermittent increases in the recruitment of the TH muscle might be associated with its reported activity in relation to changes in the pitch and the volume of vocalization.[6,8] However, in humans this role may not be consistent, since it has been reported that the TH muscle is used for this purpose by untrained singers but not by trained singers,[6] and in the dog it has been indicated that the ST muscle does not show additional activity during vocalization.[43]

The SH muscle, on the other hand, represents a quite different type of specialization. In contrast to the TH muscle, the SH muscle has the fiber type percentage composition with the greatest amount of type 2B (least fatigue-resistant) fibers. This indicates that much of the capacity of this muscle for tension generation is used for some type of infrequently occurring phasic activity.[15-18] The results reported in the physiologic literature vary with regard to the conditions under which the SH muscle is recruited. Electromyographic studies in the rabbit and rat have demonstrated phasic activity in the SH muscle during quiet breathing, with a progressive increase in activity with airway occlusion; and it has been demonstrated that the contraction of the SH muscle opposes the collapse of the upper airway from negative intraluminal pressure during inspiration.[11-13,44] However, Murakami and Kirchner[4] reported that in cats that were very lightly anesthetized, irregular discharges were present continuously throughout respiration, without any relationship to the respiratory cycle, and that during hyperpnea and inspiratory or expiratory dyspnea, there was typically no change in the discharge pattern. They also noted that when the SH muscle was shortened by a pulling down on the hyoid bone, the activity was inhibited, and that when the SH muscle was lengthened by a pulling of the hyoid bone upward, there was an increase in the frequency of spontaneously discharging potentials.[4] Ellenbogen et al[45] reported a similar absence of respiratory activity in the dog SH muscle during quiet breathing. However, they noted a very slight recruitment of the SH muscle during severe hypoxia, and they also found that when the neck was hyperextended the SH muscle displayed inspiratory activity.[44] Postural effects were reported also in more recent studies carried out by Sherrey et al,[12] using chronically implanted electrodes in unanesthetized rats during sleep. It was reported that when these animals were breathing ambient air during non–rapid eye movement (REM) sleep, the inspiratory activity of the SH muscle was increased when the animal was in a curled posture, and that carbon dioxide breathing during non-REM sleep failed to augment SH muscle inspiratory activity except in the curled posture.[12] Such complex postural and state-dependent effects may at least partially explain the inconsistent results that have been reported in physiologic studies on this muscle, although species differences may also exist.

It is unlikely that the large 2B fiber component in the cat SH muscle is recruited during quiet breathing, since the fatigue resistance of this fiber type is the lowest of the three fiber types and is probably inadequate for this constantly repetitive activity.[17-19] Furthermore, muscle fiber types are plastic, and if these fibers were recruited in connection with the constantly repetitive activity of quiet breathing, it is likely that the regulatory mechanisms that determine the fiber type would convert these fibers to one of the more fatigue-resistant types.[35,46] It is likely that this relatively large 2B fiber component in the SH muscle instead would be recruited during the occasional increases in activity that would occur with increases in SH muscle tension generation during respiratory activity caused by infrequent conditions such as postural changes, hypoxia, or hypercapnia. The type 1 fibers, and possibly also some of the 2A fiber component in the SH muscle, are probably involved in the relatively constant "postural tonic" type of activity that has been reported in electrophysiologic studies of the cat SH muscle.[4]

It should be noted also that experimental studies on the nerve-muscle pedicle procedure for reinnervation of the posterior cricoarytenoid (PCA) muscle have used the motor innervation to the SH muscle as a donor nerve on the assumption that these motor neurons have a recruitment pattern sufficiently similar to the respiratory activity of the PCA muscle and have indicated that, in the dog, reinnervation of the PCA muscle with the nerve to the SH muscle can reestablish the respiratory recruitment of the PCA muscle.[47] This finding is in apparent disagreement with that of Ellenbogen et al,[45] who reported that the dog SH muscle did not display respiratory activity during quiet breathing. More recent studies using the SH nerve-muscle pedicle in the cat have not achieved restoration of PCA muscle respiratory activity using this procedure, even when functional reinnervation has been demonstrated directly through the use of glycogen depletion techniques.[48] This result is consistent with the proportionally large 2B muscle fiber component observed in the present investigation in the cat SH muscle, since these motor units would not be expected to be recruited during the constantly repetitive activity of quiet breathing. Although species differences or postural effects might explain these apparently inconsistent findings,[12,44] it should be stressed that a clinically useful nerve-muscle pedicle procedure would require the use of a donor motor nerve having an adequate and consistent rather than posturally dependent respiratory activity unless the donor nerve was paced artificially.[49]

Finally, the ST muscle would appear to occupy a position midway between that of the SH and TH muscles with respect to the characteristics of its use. Electrophysiologic studies in the cat[4] as well as several other animal models[11,13,14,43,45,50,51] have indicated that this muscle displays phasic discharges during quiet respiration, primarily during inspiration. Furthermore, it has been demonstrated that the rabbit ST muscle shows a progressive increase in EMG activity with airway occlusion, and that the contraction of this muscle plays a role similar to that of the SH muscle in opposing the collapse of the upper airway as a result of negative intraluminal pressures during inspiration.[11] Repetitive and cyclic activity in the ST muscle would probably be based on the recruitment of type 1 and possibly also type 2A fibers, which would be adapted to resist fatigue during this constantly repetitive activity.[17-19,34,35,37,38] However, the infrequently occurring increased activity of the ST muscle in response to airway occlusion would presumably also involve the recruitment of the less fatigue-resistant 2B fibers. In addition, it has been shown that the cat ST muscle displays marked reflex discharges in response to stimulation of the superior laryngeal nerve; this finding suggests the possibilities that the ST muscle participated in the reflex closure of the larynx and that when the ST and cricopharyngeal muscles were denervated, the pressure produced by the vocal cords in response to superior laryngeal nerve stimulation was decreased.[10] The occasional increase in ST muscle activation in connection with the participation of this muscle in the reflex closure of the larynx would presumably necessitate the additional recruitment of the 2B muscle fiber type component in this muscle.

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LACK OF EFFECT OF CARBONIC ANHYDRASE INHIBITION ON DIRECT MEASUREMENTS OF ENDOLYMPH BICARBONATE

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In the past, sodium, potassium, and chloride have been measured in endolymph directly, but bicarbonate has been measured only indirectly. We sampled endolymph directly while monitoring endocochlear potentials in normal and methazolamide-treated guinea pigs. Bicarbonate was determined in samples by use of a method that depends on reduction of NADH to NAD linked to malate formation from oxaloacetate. In 11 normal animals, the bicarbonate in endolymph was 20.2 m*M* ± 4.4 m*M* (mean ± standard deviation); in six of these, plasma bicarbonate was 23.1 m*M* ± 3.5 m*M*. Nine animals treated with methazolamide (carbonic anhydrase inhibitor) had an endolymph bicarbonate of 19.5 m*M* ± 3.9 m*M*; plasma bicarbonate in five of these was 25 m*M* ± 3.2 m*M*. Carbonic anhydrase inhibition did not significantly affect endolymphatic bicarbonate levels.

KEY WORDS — bicarbonate, carbonic anhydrase, endolymph, methazolamide.

INTRODUCTION

Several diseases in humans are linked to abnormal fluid dynamics in organs, which has been linked to production of bicarbonate (eg, glaucoma, hydrocephalus).[1,2] Because the enzyme carbonic anhydrase is known to catalyze the formation of bicarbonate ion (HCO_3^-) and a hydrogen ion (H^+) from H_2O and CO_2, administration of the carbonic anhydrase inhibitors methazolamine and acetazolamide has been used to treat these disorders. It also has been used to treat Meniere's syndrome, which is thought to be related to excess endolymph. It is reasonable to suspect that endolymph production may involve carbonic anhydrase in this syndrome, as tissue concentrations in the stria vascularis are almost as high as in blood and those in the spiral ligament are higher than those in any other body tissue studied.[3,4] Also, carbonic anhydrase has been shown to be critical in fluid production in other organs, including the brain, eye, and kidney.[2] However, the most recent and best-controlled study suggests that despite the physiologic considerations, in practice acetazolamide is not effective in the treatment of Meniere's syndrome.[5] Further understanding of the normal physiology of endolymph production may help to elucidate the disordered physiology of Meniere's syndrome and suggest modes of therapy.

Levels of endolymphatic HCO_3^- in mammals have not been measured directly, but have been inferred from calculations based on endolymph pH and plasma PCO_2 to be approximately 21 m*M*.[6] The difficulties in direct measurement include 1) the minute volume of endolymph available to sample (3 μL), 2) difficulty in insuring that the fluid sampled is uncontaminated endolymph, and 3) difficulty in determining HCO_3^- in samples of 0.5 to 2.5 μL.

In this paper we describe a technique for obtaining uncontaminated guinea pig endolymph for direct measurement of HCO_3^- in samples of less than 3 μL. In addition to determining HCO_3^- in normal animals, we studied the effects of carbonic anhydrase inhibition on HCO_3^- concentration.

MATERIALS AND METHODS

Twenty guinea pigs weighing 140 to 1,050 g were used for the study. They were anesthetized with pentobarbital intraperitoneally, 18 mg/kg, and Innovar Vet intramuscularly, 0.25 mL/kg (fentanyl 0.4 mg/mL and droperidol 20 mg/mL). The animals were ventilated by spontaneous respiration through a tracheostomy. A heating blanket was used to maintain body temperature. The head was immobilized by means of a steel bolt attached to the skull and secured with acrylic dental cement. By a ventral approach through the bulla, the cochlea was exposed and the scala media was identified (Fig 1). Fenestration was created over the stria vascularis, usually in the third turn, by use of microsurgical scalpels and hand drills.

A freshly prepared double-barreled micropipette (diameter 10 to 20 μm) was directed by micromanipulator into the scala media. We fashioned the double-barreled pipette as described by I. Konishi (personal communication) by gluing together two standard glass pipettes 1.0 mm in diameter and pulling them in a modified pipette puller. One end

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REPRINTS — John H. Isaacs, Jr, MD, VA Medical Center Downtown, Dept of Surgery, Otolaryngology 112, Augusta, GA 30910.

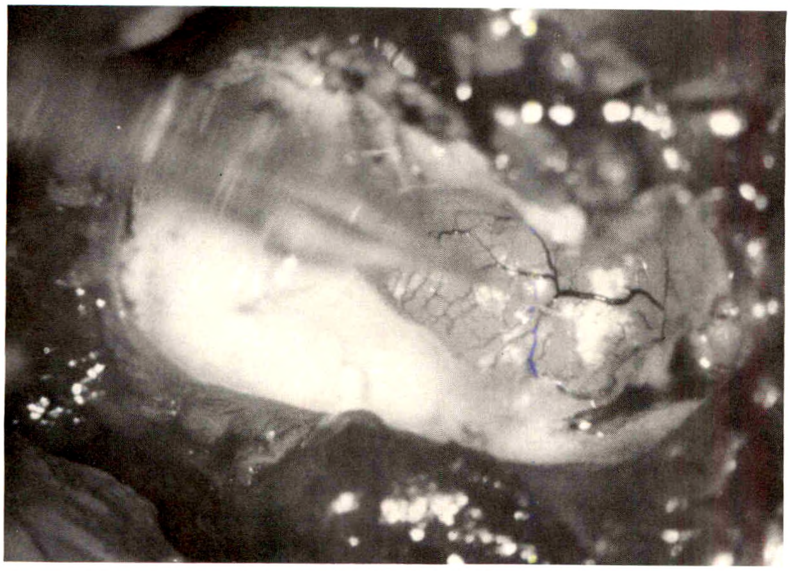

Fig 1. Photomicrograph of cochlea of 500-g guinea pig as seen ventrally, with micropipette placed in appropriate position for endolymph sampling (original x16).

of the glued pipettes was twisted 360° during pulling to form a single distal tip with two channels tightly wrapped around one another. We glued the pipettes in only one spot, allowing slight separation of their proximal ends. The glued pipettes were staggered so that the proximal ends lay approximately 2 cm apart for ease in working with the two separate channels (Fig 2).

In the shorter barrel, containing artificial endolymph solution (150 m*M* KCl and 3 m*M* NaCl), we placed a silver–silver chloride electrode that was connected to a modified Kenzie model 605 electrometer connected to a Tektronics 502 dual beam oscilloscope for measurement of endocochlear potential when this barrel was inserted in the scala media (Fig 1). In order to ensure that the samples representing endolymph were uncontaminated by perilymph, only those samples in which potentials of 25 mV or greater were maintained throughout sampling were included in the calculation of HCO_3^-.

Once an endocochlear potential was obtained, endolymph was withdrawn through the second longer barrel of the pipette while the endocochlear potential was monitored. This second collecting barrel contained a small amount of H_2O-saturated mineral oil, and after endolymph sampling, additional mineral oil was used to seal the specimen.[7,8]

We calculated the volume of fluid withdrawn by pulling the sample into the cylindrical portion of the pipette and using the standard geometric calculation $V = \pi r^2 l$ (where V = volume, r = radius, and l = length). The radius and length were measured by light microscopy. The HCO_3^- concentration was determined on 0.25 to 2.5 μL of endolymph by the method of Hall et al[9]; this method measures the oxidation of NADH by oxaloacetate, which is formed from phosphoenolpyruvate (PEP) and HCO_3^-.

$$\mathrm{PEP + HCO_3^-} \xrightarrow[\text{PEP carboxylase}]{\mathrm{Mg^{++}}} \text{oxaloacetate} + \mathrm{Pi}$$

$$\text{oxaloacetate} + \mathrm{NADH} \xrightarrow[\text{dehydrogenase}]{\text{malate}} \text{malate} + \mathrm{NAD}$$

The decrease in absorbance at 340 nm is linearly related (slope = 0.006) to the concentration of HCO_3^-. Before the animal studies were begun, the accuracy of this method was confirmed with small volumes of standard bicarbonate solution.

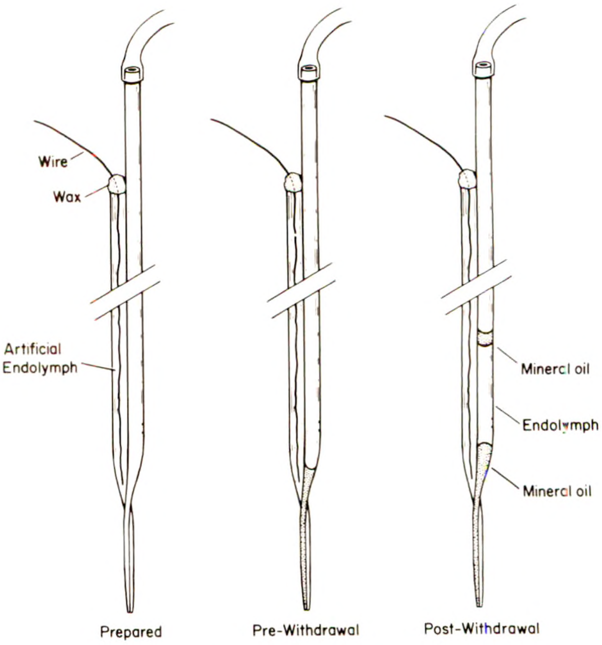

Fig 2. Various stages of preparation in use of double-barreled micropipette. At left is prepared micropipette with silver–silver chloride coated wire in artificial endolymph prior to addition of mineral oil to second chamber. Middle graphic shows micropipette prior to withdrawal of endolymph, with mineral oil in collecting barrel. At right is micropipette after endolymph withdrawal, with sample seen in cylindrical portion of collecting barrel. Volume of withdrawn sample is determined from this stage using standard geometric equation $V = \pi r^2 l$ (see text).

TABLE 1. BICARBONATE LEVELS IN ENDOLYMPH AND PLASMA OF UNTREATED ANIMALS

Animal	*Volume* (μL)	*Electric Potential (mV)*	HCO_3^- *Endolymph (mM)*	HCO_3^- *Plasma (mM)*
1	0.37	60	21.9	—
2	1.11	60	18.0	18.0
3	0.82	30	21.5	20.0
4	2.43	50	15.4	—
5	0.72	50	25.2	—
6	0.74	25	23.1	25.0
7	0.68	30	27.2	23.0
8	1.74	50	20.7	—
9	1.93	35	16.9	—
10	0.63	55	11.9	27.5
11	1.21	80	20.7	25.0
		Mean ± SD	20.2 ± 4.4	23.1 ± 3.5

Eleven animals were studied to find the normal level of endolymph HCO_3^-. In six animals, simultaneous blood samples were obtained to determine plasma HCO_3^- concentrations. Nine additional animals were pretreated with 50 mg/kg of methazolamide (pH 8.6), a potent carbonic anhydrase inhibitor, which was given intraperitoneally 1 to 14 hours before endolymph was sampled.

RESULTS

Adequate endocochlear potentials were obtained in 11 untreated normal animals. The mean HCO_3^- concentration in endolymph was 20.2 m*M* ± 4.4 m*M*. In six of these animals, the mean plasma HCO_3^- concentration was 23.1 m*M* ± 3.5 m*M* (Table 1).

Animals treated with methazolamide had a mean bicarbonate level in endolymph of 19.5 m*M* ± 3.9 m*M*. The mean plasma bicarbonate level was 25 m*M* ± 3.2 m*M* in five of these animals (Table 2). Calculation of product moment correlation revealed no significant correlation between bicarbonate concentration and time from injection of methazolamide to withdrawal. It was unexpected that plasma HCO_3^- did not fall following the administration of the drug, as a consequence of renal HCO_3^- excretion; this is the case for rat, dog, human, and many other species,[10] but has not been studied in the guinea pig.

DISCUSSION

Rigidly adhering to a 25-mV endocochlear potential as proof that the pipette tip was in endolymph also assured that the endolymph was uncontaminated; any significant contamination would have disrupted the chemical gradient of the endolymph, which acts as the generator of the endolymph potential, thus making an adequate endolymph potential unobtainable.[1] In three of the 11 normal animals the endocochlear value was 60 mV or greater, and if only such values are included, the mean remained essentially unchanged at 20.2 m*M* ± 2.0 m*M*. In three of nine methazolamide-treated animals, endocochlear potentials of 60 mV or greater were obtained, with a mean value of 20.7 m*M* ± 5.3 m*M* (also essentially unaltered). Because including the lower endocochlear potentials did not alter the results, all data were included for the sake of completeness.

The average plasma bicarbonate level was 2.7 m*M* greater than the endolymph bicarbonate level in the six control animals in which both values were obtained. In treated animals the plasma level was 5.6 m*M* greater. This difference is worthy of notice; however, it is not statistically significant ($p > .10$).

While the bicarbonate level did not significantly change after treatment with methazolamide, this does not mean that bicarbonate production was unaffected. If, indeed, fluid production is closely tied to bicarbonate production, a decrease in rate of bicarbonate production would cause a concomitant decrease in rate of fluid production; the bicarbonate concentration, on the other hand, would remain essentially the same.[11] We are currently studying this problem of fluid production further.

While the normal levels of HCO_3^- were in close agreement with the calculated values of Sterkers et al,[6] our study did not confirm their finding of a marked increase in calculated HCO_3^- level after carbonic anhydrase inhibition. Theirs is a calculated value based on the high PCO_2 (63 mm Hg) in plasma. The assumption that this is also the PCO_2 in endolymph is surely not proved.

TABLE 2. BICARBONATE LEVELS IN ENDOLYMPH AND PLASMA AFTER METHAZOLAMIDE ADMINISTRATION

Animal	*Volume* (μL)	*Electric Potential (mV)*	HCO_3^- *Endolymph (mM)*	HCO_3^- *Plasma (mM)*	*Time From Injection to Withdrawal (min)*
1	1.79	60	26.5	—	180
2	1.82	30	17.6	—	93
3	0.38	50	20.1	—	73
4	1.34	40	14.2	—	844
5	1.10	50	22.7	27	270
6	1.51	60	19.2	29	240
7	0.25	50	22.5	25	286
8	1.16	30	16.4	23	310
9	1.16	70	16.3	21	372
		Mean ± SD	19.5 ± 3.9	25.0 ± 3.2	

CONCLUSIONS

We report direct measurement of bicarbonate levels in uncontaminated endolymph. Our values for bicarbonate levels in normal guinea pig endolymph are in close agreement with those reported by Sterkers et al[6] for the indirect determination of bicarbonate. In our experiments, treatment with methazolamide before sampling did not result in bicarbonate concentration changes. We are currently investigating the rate of fluid production and whether changes are caused by methazolamide.

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THE DEAFNESS RESEARCH FOUNDATION
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The Deafness Research Foundation's Otological Research Fellowship will be sponsored by a department of otolaryngology conducting otological research. Where a unique opportunity exists in a related discipline, the fellowship may be conducted in that discipline while maintaining liaison with the department of otolaryngology. The fellowship would be scheduled as a one-year block of time at the end of the third year of medical school, thus requiring a one-year leave of absence from the medical school curriculum. Applications for 1990 must be received by November 15, 1989. For more information, please contact Walter A. Petryshyn, MD, Medical Director, The Deafness Research Foundation, 9 East 38th Street, New York, NY 10016; (212) 684-6556.

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BOTULINUM TOXIN FOR RELIEF OF BILATERAL ABDUCTOR PARALYSIS OF THE LARYNX: HISTOLOGIC STUDY IN AN ANIMAL MODEL

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We previously reported the effectiveness of botulinum toxin injections in the cricothyroid muscle under electromyographic guidance for lateralization of the true vocal cord in the mongrel dog. These additional experiments were performed to substantiate the effectiveness of botulinum toxin injections in laryngeal muscle to overcome airway obstruction produced by bilateral abductor vocal cord paralysis. A predetermined aliquot of the toxin was injected into the cricothyroid muscle of ten dogs, the duration of its effectiveness was noted, and repeated injections were given the animals. Biopsies of the injected muscles were obtained in three of the animals for routine histologic and electron microscopic studies. The effects of the toxin were recorded by cinelaryngoscopy and videotape documentation. In all the dogs, the true vocal cord was lateralized effectively and there was no morbidity, dysphagia, aspiration, or deaths. This study also confirms that the effects of the toxin in the canine larynx are spontaneously reversed and that multiple injections do not cause irreparable damage to the laryngeal muscles.

KEY WORDS — botulinum toxin, cricothyroid muscle, laryngeal obstruction, laryngeal paralysis, lateralization, true vocal cord.

INTRODUCTION

Since we reported the lateralization of the true vocal cords with botulinum toxin,[1] we failed to find in the literature any further experiments on its use to relieve obstruction of the airway from bilateral abductor paralysis of the larynx. The purpose of this study was to determine the veracity of and to confirm the findings and effectiveness of the use of botulinum toxic injected into the cricothyroid muscle to lateralize the true vocal cord. We also studied the effect of multiple injections on the muscle by histologic examination.

The search for a method of lateralizing the vocal cords in patients with bilateral abductor paralysis of the larynx is ongoing. Since the first report on the use of botulinum toxin,[1] an ingenious method was studied by Kim et al[2] on laryngeal pacemaking with use of a temperature sensor in the canine. It would seem that electrical pacemakers, which have been described, are complex and require cumbersome techniques and equipment when applied to the human. To our knowledge botulinum toxin has not as yet been used in the human larynx for countering the effects of midline paralysis of the larynx.

METHODS

The techniques we used were described in a preliminary report[1] that used the mongrel dog as an animal model. For this follow-up project, ten mongrel dogs were selected for long-term survivability after multiple injections of the toxin. Many of the dogs had been used in the first study. Because the injection was unilateral, each animal served as its own internal control for pretreatment and posttreatment position of the true vocal cords. Data were obtained by videotape documentation of the larynx of each dog prior to and immediately after injection of the toxin into the cricothyroid muscle. Further videotape documentation of the position of the true vocal cord was obtained 1 and 2 months after injection of the toxin. Still-frame analysis of the videotaped larynges proved to be an accurate means of measuring the angles formed by the true vocal cords in three predefined positions: 1) resting, 2) maximum adduction, and 3) maximum abduction. The details will be described.

Toxin Dosage. The effective dose-response curve in the mongrel dog was established in the preliminary study.[1] This was found to be 1.56 units of botulinum toxin per kilogram body weight. Appreciable variability in dose response was noted among the dogs depending on breed and/or feeding experiences. To adjust for this variation, the dose of toxin was doubled but still remained well below the intravenous LD_{50} level (median lethal dose) and was shown to be well tolerated by the dogs.

Operative Methods. Each dog was given preoperative intramuscular penicillin and was sedated with intramuscular acetyl promazine (6 mg) and atropine sulfate (0.24 mg) followed by an intravenous injection of 0.1 mg/0.4 kg of a 0.5% solution of thiopental. The dog was placed supine on an

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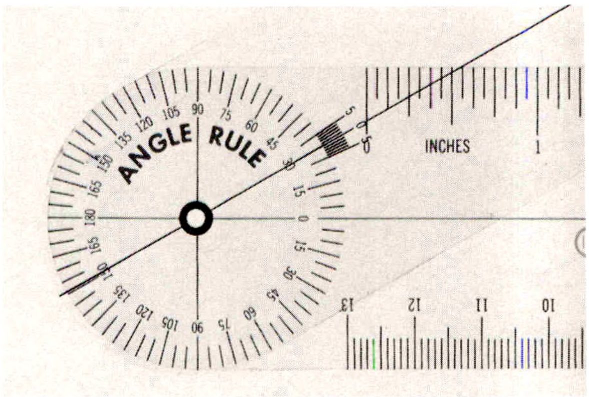

Fig 1. Goniometer with vernier scale used to measure angles formed by true vocal cords. Anterior commissure is used as axis of rotation.

operating table and a mouth gag was placed. The larynx was visualized with a rigid fiberoptic telescope and photographed with a videocamera. The laryngeal motor activity was recorded on videotape. Once preoperative videotaping of the larynx was completed, the animal was given additional thiopental to produce an operative level of anesthesia. This sedative was administered as necessary during the remainder of the experiment.

The neck of the animal was shaved, sterilized, and draped in usual fashion for the operation. A vertical incision was made in the midline of the neck over the cricoid cartilage. Self-retaining retractors were placed and the strap muscles were separated, thus exposing the cricothyroid muscle. Approximately 70 units of concentrated botulinum toxin (determined by weight of the dog) in 0.5 mL of saline was injected into the muscle through a needle attached to an electromyographic (EMG) audio amplifier. The audio signals were used for needle placement and determination of the site of toxin injection. An EMG recorder provided permanent records for later analysis of the results. Each cricothyroid muscle was probed with the needle electrode until the site of maximum electrical response was obtained. The toxin was injected into this area, which was considered to have the highest concentration of motor end plates. The wound then was closed with chromic sutures after irrigation with saline, and the wound was drained with a Penrose drain. The dogs were reexamined when awake and then were taken to a recovery cage. The operative sites were closely monitored and the drains were removed on the fourth postoperative day.

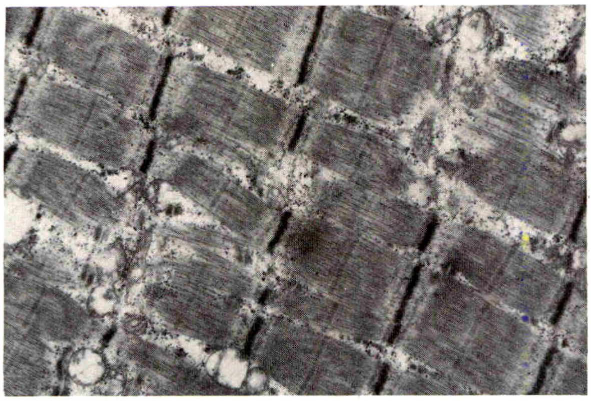

Fig 2. Electron micrograph of cricothyroid muscle 3 months after injection of botulinum toxin demonstrating normal Z bands and normal intracellular architecture.

Measurements of Effect of Toxin. The angles produced by the position of the true vocal cords were measured in three positions: 1) resting, 2) maximum abduction, and 3) maximum adduction. These measurements were taken by using the anterior commissure as the center of rotation of an arc created by the vocal cords. The degrees of the arc in which the true vocal cords rested or to which they moved were then measured using a goniometer with a vernier scale (Fig 1). To simplify the measurements, prints of the laryngeal images were obtained instantly on a video display printer.

Histologic Studies. Wedge biopsies of the cricothyroid muscle that had been injected were obtained in three randomly selected animals in order to evaluate the effect of the toxin on the muscle fibers. These specimens were stained with hematoxylin and eosin and examined by light microscopy and also unstained by electron microscopy (Fig 2).

Disposition of Animals. At the conclusion of the study, all of the dogs were returned to the veterinary unit for complete evaluation and possible adoption.

RESULTS

The ten animals did not display any intraoperative or postoperative complications. There was no significant morbidity or mortality as a result of the use of the toxin. Recovery from each anesthetic was rapid and all dogs could ambulate and feed normally within at least 2 hours or less after the procedure. There was no evidence of dysphagia, aspiration, or postoperative infection or reaction to the toxin. Several dogs developed seromas at the operative site that required needle aspiration or drainage. The effects of the toxin on the muscle activity were noted almost instantly with the injection of the toxin.

The data are shown in the Table and the mean values measuring the angulation of the true vocal cords are shown in Fig 3. Figure 4A shows the change in average cord position after toxin injection for maximum adduction, a change of 22° (note that this is a statistical representation of mean position, ie, some animals were never able to get 100% or 0° adduction even in the normal untreated state). These animals did not achieve glottic closure, yet none aspirated. A detailed examination of the videotapes shows that the false cords, arytenoids,

ANGULATION OF TRUE VOCAL CORDS BEFORE AND AFTER INJECTION OF BOTULINUM TOXIN

Dog	*Operative Measurements*	*Resting Angle (°)*	*Maximum Adduction (°)*	*Maximum Abduction (°)*
A	Pre	25	1	29
	Post 1	30	17	54
	Post 2	31	27	41
	Post 3	25	5	32
B	Pre	22	0	26
	Post 1	26	26	33
	Post 2	21	21	35
	Post 3	29	20	30
C	Pre	26	2	30
	Post 1	40	35	50
	Post 2	33	22	41
	Post 3	24	5	40
D	Pre	25	0	37
	Post 1	45	20	60
	Post 2	26	15	31
	Post 3	26	13	30
E	Pre	26	0	43
	Post 1	30	28	41
	Post 2	24	19	29
	Post 3	33	18	45
F	Pre	25	1	39
	Post 1	26	19	45
	Post 2	25	15	40
	Post 3	31	5	39
G	Pre	26	1	37
	Post 1	45	15	52
	Post 2	39	20	50
	Post 3	38	5	43
H	Pre	26	2	48
	Post 1	45	10	60
	Post 2	43	39	47
	Post 3	24	5	53
I	Pre	22	2	29
	Post 1	22	6	34
	Post 2	24	9	35
	Post 3	23	5	35
J	Pre	25	1	30
	Post 1	35	21	44
	Post 2	28	23	36
	Post 3	26	8	34

and epiglottis functioned in unison to close the larynx when stimulated by secretions. Figure 4B shows the average change after injection of the mean cord position during the resting state. The position of the cords was 15° further apart after treatment; therefore, even the resting airway was larger. The greatest change can be seen in the maximum abduction position as shown in Fig 4C. The cords after injection rested an average of 18° further apart and produced a much larger airway during inspiration. This finding indicates that the cricothyroid muscle produces about 20° of adductor closure of the true vocal cords. Thus it is evident that the toxin affected adduction twice as much as it did the resting state and 1½ times more than it affected the abducting position.

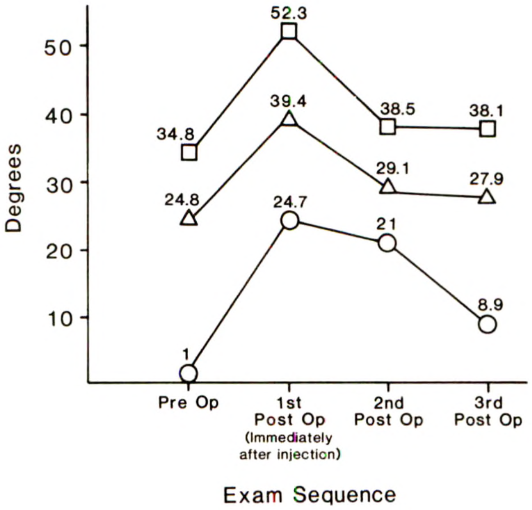

Fig 3. Comparison of mean vocal cord angle for three positions and at four different examinations associated with toxin injections. Interval between first, second, and third postoperative periods is 1 month. Circle — adduction, triangle — resting, square — abduction.

It was found that the effect of adduction lasted 30 days longer than the abduction effect or the resting position effect. In this study, the botulinum toxin was effective only for an average of 2 months. These results differ from those of the initial pilot study, in which the toxin was effective for 3 months. This difference may have resulted from deterioration of the toxin after storage for a period of 1 year. Another possibility is that the dogs had developed blocking antibodies from previous exposure to the toxin, since these dogs were used for the original dose-response curves.

Several of the dogs received four or five injections of toxin during the course of the 2-year study. No difference could be detected clinically, endoscopically, or histologically between these animals and the same animals from the beginning until the end of the study.

CONCLUSION

This second experiment was undertaken to confirm and further document the effectiveness of botulinum toxin, when injected into the cricothyroid muscle, to increase the laryngeal glottic aperture. The findings confirm that the true vocal cord indeed is lateralized with this biochemical substance, which is effective in relieving the obstructive effects of bilateral abductor paralysis of the larynx. This and our previous paper also substantiate the previous studies by Scott et al[3-7] that showed that repeated injections produce the same, albeit temporary, effects on the true vocal cord position and that the muscle suffers no damage from the toxin or the technique of its injection. Thus, paralysis of the cricothyroid muscle allows the true vocal cord to take a lateral position by

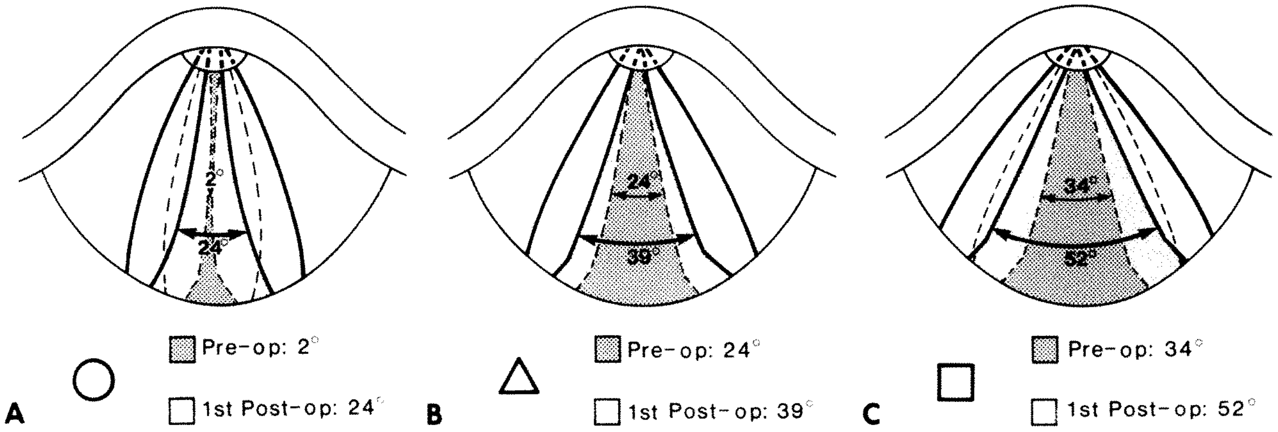

Fig 4. Representations of changes in vocal cord position after botulinum toxin injection as compared to normal. A) During maximum adduction (increase of 22°). B) At rest (increase of 15°). C) During maximum abduction (increase of 18°).

releasing tension upon the cords. The detailed pharmacologic information on the drug, the action of the drug, and the minimal pathologic effects on the muscle fibers have been described in great detail by other authors.[8-13]

Since, as has been stated before, the canine larynx most closely resembles that of the human, one can expect that botulinum toxin will affect the human larynx in a similar fashion. That the toxin's effect is temporary and relatively safe for human use already has been determined by extensive use by ophthalmologists for the treatment of strabismus. To date, there have been no reports on the use of botulinum toxin injections in the human to relieve the obstructive effects of bilateral vocal cord paralysis. The effectiveness of botulinum toxin when injected into the cricothyroid muscle to produce instant opening of the airway is established by this study.

When used as described, there was no morbidity or mortality. The complete recovery makes this methodology ideal for the human with temporary bilateral abductor paralysis of the vocal cord. It may be especially applicable to the infant with temporary paralysis of the larynx caused by birth trauma and thus may eliminate the need for an artificial airway. Histologic studies of the injected muscles showed no damage to the muscle for at least the length of time of this experiment.

It may be restated that for the otolaryngologist and specifically the laryngologist, botulinum toxin can be used to modify the laryngomechanics for at least a defined period of time with no significant danger to the larynx or the patient. The next phase would be the clinical application of the neurotoxin in the human.

While the open operative procedure was used in this animal experiment, the toxin could be injected percutaneously under EMG guidance and thus would offer minimal invasion of the dog or human.

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NATURAL HISTORY OF TREATED T1N0 SQUAMOUS CARCINOMA OF THE GLOTTIS

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The records of 373 patients with T1N0 squamous carcinoma of the glottis were examined. Of these, 271 underwent initial hemilaryngectomy; 102 had full-course irradiation for cure. While the overall survival rates of the two initial therapies were similar, failure of the initial treatment modality was approximately twice as frequent in the irradiated patients (34.4%) as in the patients undergoing hemilaryngectomy (16.9%). The effect of this difference in initial failure rate on quality of life and on cost of overall therapy will be discussed.

KEY WORDS — glottis, squamous carcinoma.

In the past, a great deal of effort has been expended both by radiation therapists and by head and neck surgeons in an attempt to identify a "proper" method of treatment for T1N0 carcinomas of the true vocal cord.[1] Issues of voice quality after treatment, recurrence rates, and final "cure rate" (usually defined as nonrecurrence prior to 5 years after initial therapy) all have been examined. In general, it has been established in many studies that both surgical treatment (via laryngofissure/cordectomy or hemilaryngectomy) and definitive radiation treatment have generally equivalent rates of "cure," as defined by 5-year survival, and that radiation therapy, by subjective measure, provides the patient with a better voice quality.[2-4] Less information is available in the literature, however, concerning the natural history of patients treated for T1N0 true vocal cord carcinoma with respect to their need for further treatment, the failure rates of initial treatment, and the overall economic cost to the patients (and to society) of the disease. It should be noted that the object of this review is not to determine a "best" treatment for T1N0 carcinoma of the true vocal cord, but is simply to focus on factors that may become more important in the current atmosphere of medical treatment, and that should be considered when choosing a "best" solution for an individual patient or group of patients.

The Washington University Tumor Registry is a computerized data base consisting of the records of approximately 4,800 patients treated for head and neck cancer who have presented themselves to the Department of Otolaryngology at Washington University over the past 35 years. Examination of the records of those patients who within the last 15 years (1972 to 1987) underwent biopsy and were judged to have squamous carcinoma of a single, mobile true vocal cord was performed. For this study, the Tumor Registry data were analyzed with several questions in mind.

Of the 489 patients with a recent diagnosis of epidermoid cancer of a single vocal cord, 54 were lost to follow-up and therefore were excluded from further study. Of the remainder, 373 had cancer staged (AJC 1972) as T1N0. Of these, 271 received hemilaryngectomy and 102 received a median of 6,300 rad of external beam irradiation as primary, curative treatment (Table 1). Mean follow-up was slightly more than 7 years for those patients initially treated with radiation therapy, and slightly greater than 9 years for those treated initially with surgery (Table 1).

The demographics of these populations were quite similar in duration of symptoms prior to treatment and age at onset of symptoms (Table 1), and differed little in tobacco and ethanol use. Sex ratios were approximately 9:1 male to female in both groups of patients (Table 1).

At the time of last follow-up, 52 of 102 patients initially treated with radiation therapy were still

TABLE 1. PATIENT DATA BY MODE OF TREATMENT

	Hemilaryngectomy	*Irradiation*
No. of patients	271	102
Mean follow-up (mo)	112.6	84.9
SEM	4.2	7.4
Mean duration of symptoms (mo)	9.7	8.8
SEM	0.92	1.6
Mean age (yr)	61.5	61.3
SEM	1.2	0.64
Male (No.)	244	90
% (per procedure)	91	88
Female (No.)	27	11
% (per procedure)	9	12

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TABLE 2. FINAL TREATMENT RESULTS

	Hemilaryngectomy		*Irradiation*	
	Pts	*%*	*Pts*	*%*
Alive	151	56.0	52	51.0
Dead — primary uncontrolled	4	1.5	9	8.8
Dead — neck uncontrolled	6	2.2	0	0.0
Dead — primary neck disease	4	1.5	0	0.0
Dead — distant metastasis	7	2.6	3	2.9
Dead — treatment complication	3	1.1	0	0.0
Other causes of death	96	35.0	38	37.0

alive (51%). Nine had died of problems arising from uncontrolled primary vocal cord cancer, and three had died of distant metastasis, presumably from the initial laryngeal source. The remainder had died of causes not directly related to the initial laryngeal tumor (Table 2). Of the surgically treated patients, 151 of 271 were still alive (56%). Four had died of problems arising from uncontrolled primary cancer, six of neck disease, four of a combination of an uncontrolled primary and neck disease, seven of distant metastasis, and three of complications of treatment (Table 2). These figures give overall death rates as a result of overall treatment failure of 9.0% and 11.8%, respectively, for surgical and irradiative treatment.

Voice quality was assessed on a very subjective scale of good, fair, and poor; it appeared from the data available on living patients and from patients whose voices had been functional before death that it was approximately 1.5 times as likely that an examiner would judge a patient to have a "good" voice after radiation therapy as after hemilaryngectomy. Interestingly, however, it appeared that the number of patients after either treatment who were judged to have a "poor" voice was approximately equal when weighted for the size of the cohort (Table 3). Voice quality judgments were made only after initial treatment and did not include those patients in whom, for instance, a total laryngectomy eventually was required after initial treatment with radiation therapy yielded a "good" voice.

Differences existed in the natural history of the cancer after initial treatment that are not evident from the final survival figures. In the irradiated group of 102 patients, 21 had a recurrence or persistence requiring further treatment within 3 years of their initial therapy. Seven more had a recurrence within an additional 2 years, and seven more after 5 years from the time of their initial irradiation for cure. In patients initially treated with hemilaryngectomy, there were 23 recurrences within 3 years of follow-up, nine more after another 2 years, and 14 more after more than 5 years after initial treatment. This gives an overall rate of initial failure with surgical treatment of 16.6%, and with irradiative treatment of 34.3% (Table 4).

TABLE 3. VOICE QUALITY AFTER TREATMENT

	Hemilaryngectomy		*Irradiation*	
	Pts	*%*	*Pts*	*%*
Good	74	42	35	59
Fair	39	22	3	5
Poor	21	36	64	36

TABLE 4. TIME FROM INITIAL TREATMENT UNTIL FAILURE

	Hemilaryngectomy		*Irradiation*	
Years Until Failure	*Pts*	*%*	*Pts*	*%*
<3	23	8.5	21	20.6
3-5	9	3.3	7	6.9
>5	14	5.2	7	6.9
Overall initial failure	46	16.6	35	34.3

In the group initially irradiated for cure, 16 of the 35 patients who experienced a recurrence or persistence eventually required total laryngectomy for salvage. However, 25 patients received total laryngectomy in an attempt to preserve their lives after they had a recurrence or persistence. In the group initially treated with surgery, 15 of the 44 patients with a recurrence or persistence eventually required total laryngectomy for salvage, while 21 had a total laryngectomy in an attempt to salvage their lives. The remainder of the patients received no effective treatment or were treated with initial or additional radiation therapy (Table 5).

In this preliminary analysis, we have looked at several factors that have been examined in less detail in prior studies of the natural history of T1N0 glottic cancer.[5-8] Recurrence rates appear to depend on the choice of initial treatment, and in this series recurrence rates, especially for radiation therapy, appear higher than in many prior studies.[3,8-12] The reason for this is unclear. It is interesting to note that the salvage rates, as defined by the cure of patients after recurrence, was apparently lower among the population treated initially with surgery. However, the overall cure rates of both initial treatments were similar, because of the lesser need for secondary treatment among patients initially treated with surgery. Overall cure rates were in the range of values previously reported.[13-15] It is interesting to note in this study that after all treatment had been completed, a slightly greater percentage of "cured" patients initially treated with radiation therapy had no larynx. This finding is in contradiction to other studies that have noted very high salvage rates with hemilaryngectomy alone.[13-15]

TABLE 5. RECURRENCE AND SALVAGE

	Hemilaryngectomy		*Irradiation*	
	Pts	*%*	*Pts*	*%*
Recurrences	46		35	
Salvaged	24	52	23	66
Required total laryngectomy for attempted salvage	21		25	
Required total laryngectomy for successful salvage	15		16	

TABLE 6. ESTIMATED RELATIVE COSTS OF TREATMENT

	Irradiation	*Hemilaryngectomy*
Initial cost	1.00	1.28
Attempted salvage cost	0.43	0.15
Average cost of treatment	1.43	1.42

Follow-up in these studies, however, was not generally as long as in the present one.

Using these data on the natural history of T1N0 carcinoma of the true vocal cord, it is interesting to compare relative costs of overall treatments that begin with radiation or with surgery. Typically, a course of radiation therapy suitable for treatment of a T1N0 carcinoma of the true cord costs approximately two thirds to three fourths as much as initial treatment with hemilaryngectomy, including surgeon's fees, hospitalization, and postoperative care. The monetary cost of successful patient salvage, in the face of recurrence, is approximately equal. However, when the cost of unsuccessful attempts to salvage life also is added to each treatment regimen, again basing cost on the probable need for treatment in each cohort, initial treatment of the patient by hemilaryngectomy becomes economically slightly more desirable than initial treatment with radiation therapy (Table 6), because of the fact that it is more likely that patients undergoing initial radiation therapy in this series will have a recurrence. Several factors, such as lost wage-earning capacity as an opportunity cost, and lost quality of survival, either were not factored into this preliminary analysis or were extremely difficult to quantify objectively. However, given the present climate of medical practice, analyses such as these will become important as measures of patient performance and of cost of a given treatment to individual patients.

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EFFECT OF VOCAL FOLD AUGMENTATION ON LARYNGEAL VIBRATION IN SIMULATED RECURRENT LARYNGEAL NERVE PARALYSIS: A STUDY OF TEFLON AND PHONOGEL

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Flaccid laryngeal nerve paralysis may be treated by vocal fold augmentation with Teflon injection, which is successful to various degrees depending on the subjective interpretation of the patient or clinician. A new material, Phonogel, consisting of cross-linked bovine collagen, is available but not approved for human use in this area. Ten dogs were submitted to videostroboscopy, photoglottography, electroglottography, and acoustic analysis in the normal state, with simulated recurrent laryngeal nerve paralysis, and with injection of either Teflon or Phonogel. A statistical comparison and the advantages and disadvantages of each material are discussed in relation to this study and its clinical use.

KEY WORDS — flaccid laryngeal nerve paralysis, Phonogel, recurrent laryngeal nerve, Teflon, vocal fold injection.

INTRODUCTION

Paralysis of the human vocal folds is a problem frequently encountered by otolaryngologists. Usually a unilateral paralysis is encountered and the majority of these are caused by injury to the vagus or recurrent laryngeal nerve by surgery or an adjacent tumor.[1]

For most patients with unilateral vocal fold paralysis, compensation takes place over a period of several months, allowing resumption of acceptable communication, absence of aspiration, and an effective cough. For those patients whose laryngeal dysfunction does not improve, a variety of methods have been developed to treat unilateral vocal fold paralysis. Reinnervation techniques focus on providing a neural or neuromuscular transplant to the affected lateral cricoarytenoid muscle—the primary adductor of the vocal fold.[2,3] Although these techniques have potential for reinnervating the larynx, they have not had widespread acceptance. In contrast, most currently used methods rely on passive techniques to medialize the paralyzed vocal fold. Methods of passive medialization rely on an intact separate innervation to the contralateral vocal fold that when tensed can abut the ipsilateral paralyzed, but now medialized, vocal fold. One method, thyroplasty type I, uses thyroid cartilage as an autograft placed in a pocket developed between the thyroid ala of the affected fold and the fold itself.[4] Other methods of medialization by augmentation rely on the use of inert substances such as Gelfoam,[5] glycerin,[6] and Teflon.[7-9] The last substance has been the mainstay of treatment, as it seems to be the most easily used and produces the most predictable results. Recently, several investigators have reported preliminary results in treating vocal fold paralysis with bovine cross-linked collagen (Phonogel, Collagen Corp, Palo Alto, Calif).[10-12]

Regardless of which form of surgical treatment is used, results vary according to subjective evaluation of phonation and glottic competency. In fact, among physicians trained in laryngology it is not uncommon to have differing opinions regarding the outcome of these treatments in individual patients. In addition, a single otolaryngologist may have difficulties in assessing the results of vocal fold augmentation over time.

Several investigators have used various objective measures in human subjects to evaluate the effect of Teflon augmentation on vocal fold paralysis. Rontal et al[13] used voice spectrography to monitor the performance of vocal fold Teflon injection procedures. Cormier et al[14] measured forced inspiratory and expiratory airflows in patients before and after Teflon injection. Rubin[15] demonstrated the vibratory characteristics of the vocal folds by high-speed photography both preinjection and postinjection. Von Leden et al[16] used several objective measures of laryngeal function in addition to acoustic analysis of the paralyzed and Teflon-injected states.

Currently, a number of objective tests may be used as aids in diagnosis and therapy of dysphonias. Photoglottography (PGG) is a method that measures the amount of light transmitted through the vocal folds during phonation. Typically, a light

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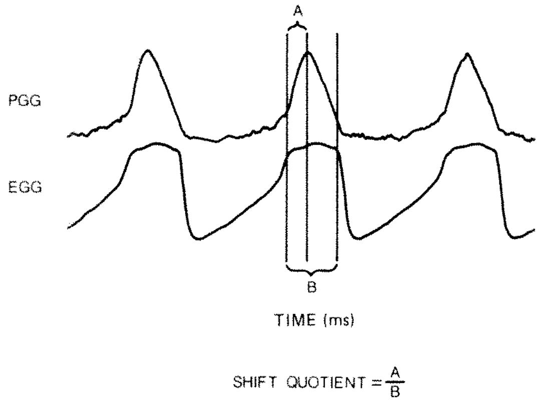

Fig 1. Calculation of shift quotient in dog with phonation produced in normal state. PGG — photoglottographic waveform, EGG — electroglottographic waveform.

source is placed in the mouth or pharynx while a photosensor is placed on the neck below the folds. As the vocal folds vibrate, changes in the glottal area produce proportional changes in the intensity of light transmitted. It has been shown by comparison with high-speed photography that the intensity of the transmitted light during PGG is a good approximation of cross-sectional area of the glottic aperture during phonation.[17-19]

Another technique, electroglottography (EGG), measures change in electric impedance across the larynx as the vocal folds vibrate. Comparison between EGG and stroboscopic investigation of the vibrating vocal folds substantiates the view that the EGG signal reflects variations of lateral vocal fold contact area.[20] Baer et al[19] stated that EGG and PGG are complementary measures, in that the former provides information about glottic closure, while the latter helps describe glottic opening.

Two measures of the glottic cycle with potential clinical application that can be calculated by use of PGG and EGG waveforms are the open quotient (OQ) and the speed quotient (SQ). Open quotient is defined as the fraction of time the folds are open during the glottic cycle. Speed quotient is the duration of time the folds are opening divided by the duration of time the folds are closing. Trapp and Berke[21] recently presented a new measure, the shift quotient (ShQ), that may be valuable in identifying flaccid laryngeal paralysis (Fig 1). Each of these measures, OQ, SQ, and ShQ, holds potential as an objective measure for judging the outcome of various treatments for vocal fold paralysis.

Another objective though less quantifiable measure of vocal fold vibration is videostroboscopic laryngoscopy. Recent miniaturization of videocameras combined with available fiberoptic systems has created a tremendous clinical interest in this form of documentation. Moore et al[22] recently studied videolaryngoscopy in an in vivo canine model of phonation with the addition of stroboscopy to detail the unique characteristics of normal vocal fold vibration in addition to the vibratory characteristics of recurrent laryngeal, superior laryngeal, and vagal nerve paralyses.

Although laryngologists have been using Teflon to augment vocal folds for the past 20 years, the optimal amount of Teflon to inject into the vocal fold has not been determined by any objective measurement. Most experienced laryngologists have estimated a quantity in the range of 0.2 to 0.5 mL. It became apparent to us that photoelectric techniques and videostroboscopy might be useful in determining the amount of synthetic material needed for return to optimal pre-paralysis vocal function.

Ten adult male mongrel dogs were studied by EGG and PGG to compare the effects of Phonogel and Teflon augmentation on simulated unilateral recurrent laryngeal nerve paralysis. This was performed according to a previously reported in vivo canine model of phonation.[23] Photoelectric data and videostroboscopic images of laryngeal vibration were obtained for normal phonation, simulated recurrent nerve paralysis phonation, and postinjected states—both Teflon and Phonogel. This study reports the results obtained.

MATERIALS AND METHODS

Operative/Photoelectric. Ten adult male mongrel dogs were premedicated with Innovar intramuscularly. Intravenous pentothal was administered to a level of corneal anesthesia and additional pentothal was used to maintain this level of anesthesia throughout the experiment.

The animals were placed supine on the operating table, the neck was shaved, and a midline incision was made to expose the trachea from the hyoid to the sternal notch. Both recurrent laryngeal nerves were identified and preserved. Both superior laryngeal nerves were identified just lateral to their entrance into the cricothyroid muscles. Figure 2 is a schematic representation of the experimental setup. A low tracheotomy was performed at the level of the suprasternal notch, through which an endotracheal tube was passed to allow spontaneous respirations. A second tracheotomy was performed in a more superior location, through which a cuffed endotracheal tube was passed in a rostral direction for several centimeters and positioned 10 cm below the vocal folds. The cuff was inflated to just seal the trachea. Humidified heated air was passed subglottically through this second endotracheal tube from a compressed air canister, and flow was controlled with a valve and flowmeter. The warmth and humidification of the air were provided by bubbling the air through 5 cm of heated water to provide a constant temperature of 37°C as measured at the laryngeal lumen. A 1-cm button was used to suspend the epiglottis from a fixed point to provide

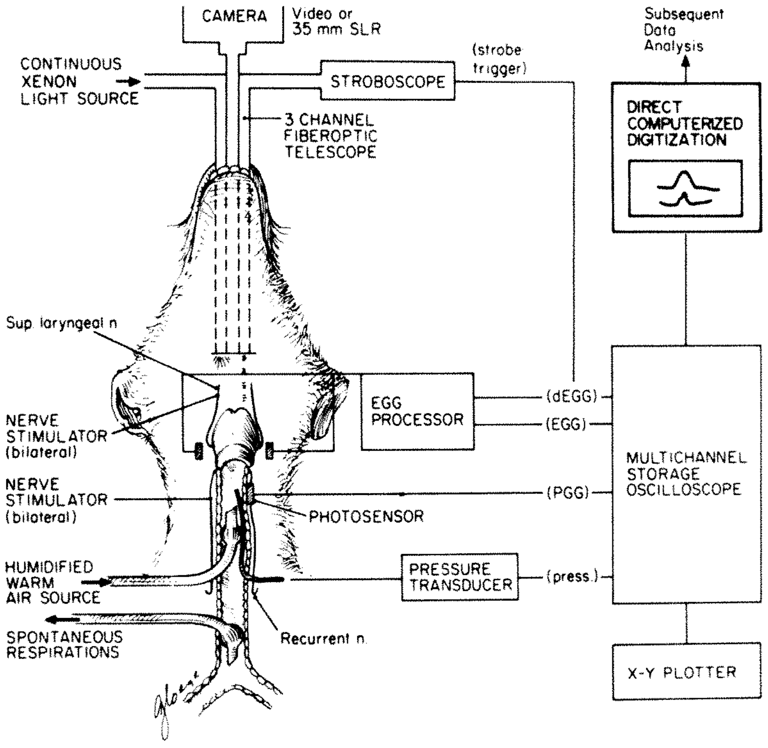
CAMERA
Video or 35 mm SLR
CONTINUOUS XENON LIGHT SOURCE
STROBOSCOPE
(strobe trigger)
3 CHANNEL FIBEROPTIC TELESCOPE
Subsequent Data Analysis
DIRECT COMPUTERIZED DIGITIZATION
Sup laryngeal n
NERVE STIMULATOR (bilateral)
NERVE STIMULATOR (bilateral)
EGG PROCESSOR
(dEGG)
(EGG)
(PGG)
PHOTOSENSOR
MULTICHANNEL STORAGE OSCILLOSCOPE
HUMIDIFIED WARM AIR SOURCE
PRESSURE TRANSDUCER
(press.)
SPONTANEOUS RESPIRATIONS
Recurrent n.
X-Y PLOTTER

Fig 2. Schematic representation of experimental setup for in vivo canine model of phonation. SLR — single lens reflex, EGG — electroglottography, dEGG — differentiated electroglottography, PGG — photoglottography.

direct visualization of the larynx through the oral cavity.

A 1-cm segment of the recurrent and superior laryngeal nerves was isolated and Harvard silver electrodes were applied around the nerve. The electrodes then were insulated from the surrounding tissue. Two nerve stimulators (Grass Instruments) with direct current isolation units were used to stimulate the recurrent and superior laryngeal nerves. These nerves were stimulated at 70 to 90 Hz stimulus frequency, with 2 to 7 mA intensity for 1.5 ms pulse duration. Phonation was produced with airflow of 375 mL/s supplied through the laryngeal aperture by the rostrally placed endotracheal tube while the laryngeal nerves were being stimulated.

Changes in glottic area were monitored by PGG. The larynx was illuminated from above with a fiberoptic xenon light source secured in the oral cavity and directed toward the glottis. Light transmission through the vocal folds was transduced with a photosensor (Centronics OSD 502) placed on the trachea just below the cricoid cartilage. Electroglottographic signals were obtained with a laryngograph (Synchrovoice) with the two recording electrodes placed in pockets just above the cricothyroid muscles adjacent to the thyroid cartilage. The ground electrode was secured to adjacent strap muscles. Photoglottographic and EGG tracings were observed on a storage oscilloscope (Tektronix 5116) and directly digitized and stored onto the hard disk of a personal computer (80286 microprocessor).

Acoustic data were acquired with a highly sensitive Bruel & Kjaer condensor microphone placed at a constant distance (30 cm) from the glottis and were stored on the hard disk of a personal computer.

Computerized Data Digitization, Storage, and Analysis. Analog data from EGG, PGG, and acoustic signals were low-pass filtered at 3,000 Hz and digitized real-time at 20,000 Hz by use of the personal computer equipped with a math coprocessor (80287) and a commercial software program (C-Speech, Paul Milenkovic, PhD, University of Wisconsin). The EGG and PGG waveforms obtained were examined briefly after each trial of phonation to evaluate the adequacy of the waveforms. Three seconds of waveform data was stored on the computer's hard disk and later recovered for analysis and calculation of the OQ and SQ. In addition, the ShQ was derived from the EGG and PGG waveforms (Fig 1). This was done by calculating the distance from the beginning of the plateau phase of the EGG to the apex of the PGG as it was superimposed directly on the EGG and dividing this by the duration of the plateau phase of the EGG (Fig 1). Points of glottic opening and closing were determined in the manner previously described by Childers et al.[24] Vocal fold opening was marked at the peak of the differentiated EGG waveform, and closing at the nadir of the differentiated EGG waveform.

Data were analyzed in the following format. Within each trial of simulated phonation a stable

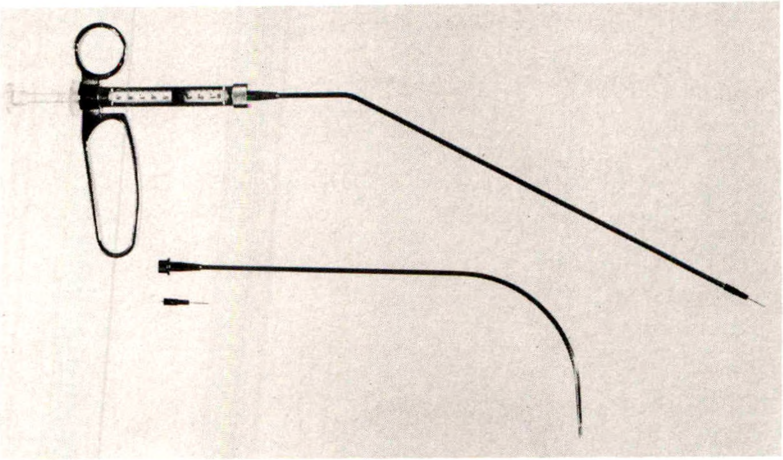

Fig 3. Phonogel injector apparatus with 26-gauge needle and single-use syringe of Phonogel in position.

0.25-second portion was chosen in four equally separated parts of the 3-second recording. Within these 0.25-second segments, five consecutive waveforms were analyzed interactively and averaged to calculate a single value for OQ, SQ, and ShQ. The values then were compared with those obtained in the three other 0.25-second segments and a mean value was calculated and recorded as the OQ, SQ, and ShQ for each simulated phonation. The values of each of three trials were compared and averaged so that each dog had one value for OQ, SQ, and ShQ.

Signals also were examined qualitatively to determine how their overall shape related to the vibratory patterns seen on videostroboscopy.

Available software was used to obtain the fast Fourier transformation from the acoustic signals (C-Speech).

Video Imaging. A Storz laryngostrobe unit (model 8000) was used for stroboscopic video imaging that was performed with each trial of phonation after the photoelectric data had been recorded. The stroboscope was connected to a Storz 0° telescope via a fluid-filled light cable. The image from the 0° scope was recorded on a Storz CCD (charge-coupled device) video camera (model 9000) and a Sony U-matic videocassette recorder (VO-5800). The video images subsequently were analyzed frame-by-frame by use of the same videorecording unit.

Injection of Teflon/Phonogel. Injections were carried out by use of fiberoptic laryngoscopy. Five dogs each were injected with Teflon and Phonogel, respectively. Teflon was injected in the standard method as reported by Arnold[7] with a Breuning's syringe. This injection was placed so that the Teflon was lateral to the vocalis muscle. Phonogel was injected with a specially designed injector (Collagen Corp, Palo Alto, Calif) (Fig 3). This injection was performed within the submucosa just deep to the mucosal plane as described by Ford et al.[10]

Increments of 0.1 mL of Teflon or Phonogel injection were analyzed stroboscopically and by photoelectric waveform analysis during each experiment. The end point of Teflon or Phonogel augmentation was considered to have been reached when vocal fold vibration most closely appeared as a travelling mucosal wave. Several animals then underwent overinjection of Teflon or Phonogel and stroboscopic images and photoelectric signals were obtained.

Experimental Design. Recordings were taken for a minimum of three trials of each state: normal (simultaneous bilateral recurrent and superior laryngeal nerve stimulation), unilateral recurrent laryngeal nerve paralysis, and postinjection/augmentation (Phonogel or Teflon).

A constant low level of bilateral cricothyroid tension (0.2 mA stimulation) was maintained in all ten dogs. The recurrent laryngeal nerves then were stimulated bilaterally until phonation ensued to provide data for normal phonation. These ten dogs

TABLE 1. QUOTIENT VALUES FOR TEFLON GROUP

Dog	*Normal*	*Paralysis*	*Teflon Injection*
Open quotient			
1	0.22	0.30	0.31
2	0.30	0.52	0.42
3	0.23	0.42	0.28
4	0.30	0.39	0.30
5	0.25	0.31	0.31
Mean	0.26	0.39	0.32
Speed quotient			
1	1.14	1.01	1.15
2	0.92	0.92	0.96
3	1.02	1.03	0.86
4	1.13	1.19	1.06
5	1.19	0.60	1.02
Mean	1.08	0.95	1.01
Shift quotient			
1	0.76	0.38	0.57
2	0.51	0.29	0.60
3	0.68	0.38	0.73
4	0.62	0.47	0.55
5	0.65	0.51	0.79
Mean	0.64	0.41	0.65

TABLE 2. QUOTIENT VALUES FOR PHONOGEL GROUP

Dog	*Normal*	*Paralysis*	*Phonogel Injection*
Open quotient			
6	0.19	0.48	0.31
7	0.22	0.42	0.40
8	0.36	0.65	0.53
9	0.27	0.29	0.17
10	0.13	0.51	0.34
Mean	0.23	0.47	0.35
Speed quotient			
6	1.07	0.63	0.61
7	1.10	1.21	0.95
8	1.00	1.32	1.50
9	0.69	1.03	1.16
10	1.74	1.09	1.08
Mean	1.12	1.06	1.06
Shift quotient			
6	0.47	0.36	0.45
7	0.56	0.33	0.49
8	0.53	0.29	0.31
9	0.56	0.31	0.63
10	0.73	0.33	0.67
Mean	0.57	0.32	0.51

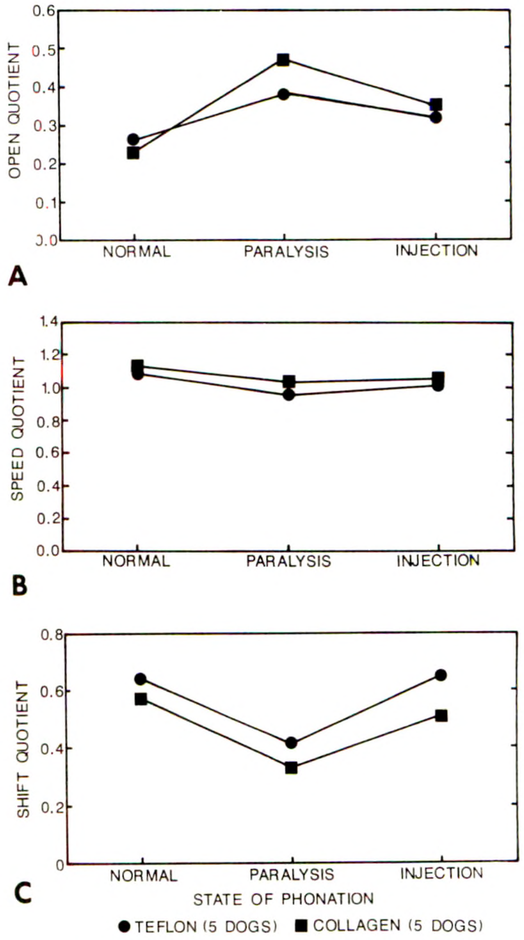

Fig 4. Mean values of A) open quotient, B) speed quotient, and C) shift quotient.

then each underwent trials of phonation with one recurrent laryngeal nerve transected and stimulation to both superior laryngeal nerves and the remaining recurrent laryngeal nerve. The dogs then were divided into two groups. The first group of five dogs was treated with Teflon augmentation of the vocal fold on the side of the transected recurrent laryngeal nerve. The second group of five dogs had the paralyzed fold augmented with Phonogel.

Statistical Analysis. Data for each quotient group were subjected to a 2 x 3 mixed-factor analysis of variance (ANOVA), substance by phonation, respectively. In addition, post hoc Newman-Keuls testing was used to compare states of phonation for the OQ and ShQ values.

RESULTS

Photoelectric Data. A mean value for OQ, SQ, and ShQ was calculated for each of the ten dogs in each experimental cell—normal phonation, phonation with unilateral recurrent laryngeal nerve paralysis, and phonation after augmentation with Teflon or Phonogel. Tables 1 and 2 depict the mean quotient values for each treatment cell.

Figure 4A compares the mean values for OQ—Teflon versus Phonogel. As seen from the graph, the preinjection states were similar, ie, there was no main effect for treatment group on OQ. Further, there was no interaction between treatment group and state of phonation. There was however, a significant main effect for state of phonation attributable to the paralyzed condition: $F(2,16) = 17.75$, $p < .001$. A post hoc Newman-Keuls test was applied and showed that the paralyzed condition was significantly different from normal ($p < .05$) and that paralyzed and injected states were similar. In addition, normal and injected states were significantly different ($p < .05$). In other words, neither group of dogs recovered to "normal" values and the injected dogs were not statistically different from those in the paralyzed state. In addition, there was no statistically significant difference in the performance of Teflon when compared to Phonogel for changes in OQ.

Figure 4B compares the mean values for SQ. No statistically significant difference was observed between the Teflon and Phonogel groups, and no difference between the normal, paralyzed, and postinjected states.

Figure 4C compares mean values for ShQ. There was no main effect for treatment group on ShQ and there was no interaction between treatment group and state of phonation. There was a significant main effect for state of phonation attributable to the paralyzed condition: $F(2,16) = 25.86$, $p < .001$. Post hoc testing was applied and showed that the paralyzed condition was significantly different from normal ($p < .05$) but that normal and injected states were not significantly different from one another.

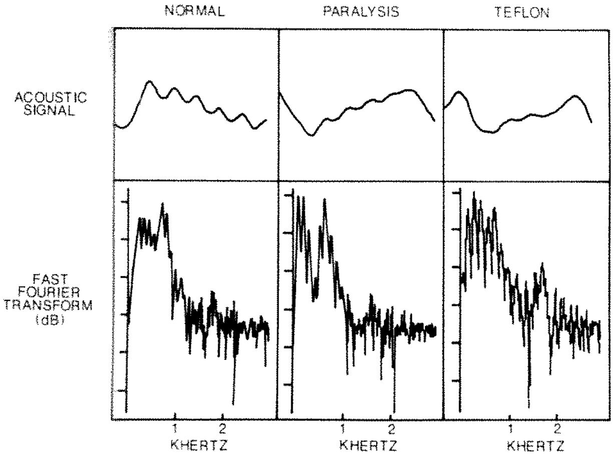

Fig 5. Sample of acoustic waveforms and fast Fourier transformation analysis of single dog in normal, recurrent laryngeal nerve paralysis, and post-Teflon injection states.

Videostroboscopy. Videotapes of each of the ten dogs in each of the two preinjected states of phonation (normal and recurrent nerve paralysis) were compared to the vibratory patterns of the postinjected states (Teflon and Phonogel). As previously reported by Moore et al,[22] a symmetric travelling mucosal wave normally occurs from the lower to the upper fold margin as the glottic air puff is released. The characteristic findings with unilateral recurrent laryngeal nerve paralysis were loss of the travelling mucosal wave bilaterally as the glottic air puff was released and a homogeneous lateral excursion to the paralyzed vocal fold (one mass). The nonaffected vocal fold remains tense near the midline with little excursion.

After minimal augmentation of the paralyzed fold with Teflon, a return toward the normal two-mass (upper and lower margin) model of oscillation with resumption of the travelling mucosal wave to the nonparalyzed fold was noted. With further augmentation this effect was enhanced.

With overinjection it was observed that the paralyzed flaccid fold became the tensed vocal fold and that the normal nonparalyzed fold vibrated in a manner similar to that of the previously paralyzed fold. The same was true for dogs overinjected with Phonogel. A videotape summarizing the results was presented recently.[25]

Acoustic Analysis. Of interest was the effect of laryngeal paralysis and vocal fold augmentation on the acoustics of phonation. Figure 5 depicts the acoustic signal and fast Fourier analysis of the acoustic signal for normal, paralyzed, and Teflon-injected states of phonation. All three phonation states are characterized by a strong formant visible at about 800 Hz. The paralyzed state shows a sharp drop-off in the amplitude of the third and fourth harmonics due to the breathy phonation. Teflon injection to the point at which the ShQ was corrected to normal shows a resumption in harmonic amplitudes. This also was associated with a perceptual increase in the acoustic intensity of phonation.

With simulated normal phonation a change in fundamental frequency (F0) was related to changes in recurrent laryngeal nerve stimulation. In the paralyzed state there was no change in F0 associated with changes in recurrent laryngeal nerve stimulation because of loss of subglottic pressure modulation. Vocal intensity was not measured in each case, but perceptual decrease in intensity was noted in the paralyzed state and was associated with the decrease in harmonics seen in Fig 5.

DISCUSSION

Open quotient, a relative measure of the time period the glottis is open as a function of the entire glottic cycle, has been found to increase with flaccid laryngeal paralysis of the recurrent nerve type.[26] The clinical correlate is observed when the patient has a weak and breathy voice. This value would be expected to decrease toward normal with augmentation by either Teflon or Phonogel, as it did, though not to a degree that was statistically significant.

Speed quotient was not found to alter significantly in this study and in similar studies.[21]

We have found the ShQ to be a useful measure in the identification of flaccid laryngeal paralysis. A shift of the peak of the PGG to the left in relation to the EGG occurred because the paralyzed flaccid vocal fold vibrates in a one-mass mode. This implies no phase lag between the opening of the lower margin and upper margin excursion. The homogeneous lateral excursion of the paralyzed fold caused the vocal fold aperture to be at its maximum (ie, peak of PGG) when the cord unzips completely in the horizontal plane (ie, plateau phase of EGG). It is possible to measure this shift in the apex of the PGG with respect to the beginning of the plateau phase of the EGG (length A, Fig 1). We quantitate its relative value by using the duration of the EGG plateau phase as the denominator (length B, Fig 1).

Figure 6A,B shows the contrast in ShQ typically seen for dogs with normal phonation and dogs with unilateral recurrent nerve paralysis, respectively. Figure 6C shows that with Teflon augmentation, the peak of the PGG is shifted back into a later portion of the EGG plateau phase, as seen in normal phonation. Figure 6D depicts similar findings in another dog when a paralyzed vocal fold was injected with Phonogel. This shifting of the PGG peak relative to the EGG plateau phase has been quantified, and when statistically compared, the normal and injected states were not found to be statistically different.

The morphology of the photoelectric waveforms also changed with augmentation. With recurrent

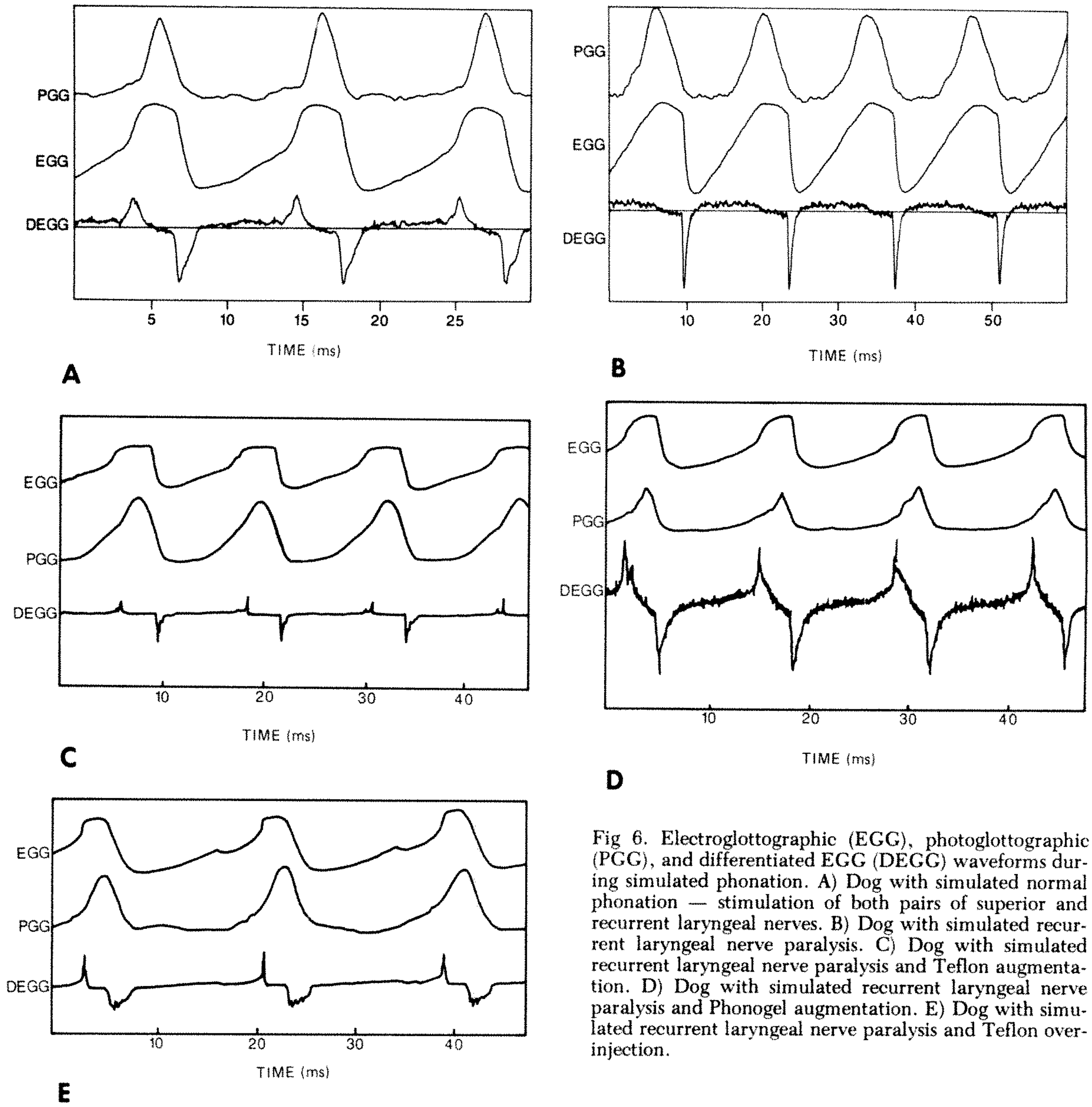

Fig 6. Electroglottographic (EGG), photoglottographic (PGG), and differentiated EGG (DEGG) waveforms during simulated phonation. A) Dog with simulated normal phonation — stimulation of both pairs of superior and recurrent laryngeal nerves. B) Dog with simulated recurrent laryngeal nerve paralysis. C) Dog with simulated recurrent laryngeal nerve paralysis and Teflon augmentation. D) Dog with simulated recurrent laryngeal nerve paralysis and Phonogel augmentation. E) Dog with simulated recurrent laryngeal nerve paralysis and Teflon overinjection.

nerve paralysis the slope of the opening phase of the EGG waveform usually failed to demonstrate the discontinuity that represents the normal transition from vertical to horizontal fold separation. This loss of change in EGG slope is due to the simultaneous opening of the upper and lower vocal fold margins that results when the two-mass movement is lost (compare Fig 6A and B). After injection of the paralyzed fold a hint of the two-mass morphology reappears, as there is discontinuity in the waveform during the opening phase of the glottic cycle (see PGG wave in Fig 6C).

Videostroboscopy confirmed the return to a more normal two-mass model of vocal fold vibration after treatment with either Teflon or Phonogel. There were no obvious performance differences between the two substances that supported the use of one of these synthetic agents over the other. We did note on careful review that a noticeable degree of augmentation achieved with Phonogel was lost over a period of time, sometimes as short as 30 minutes. This is apparently a function of the high water content of Phonogel in the cross-linked bovine form. Overinjection was needed in the initial treatment to attain a degree of vocal fold closure that could be maintained throughout the experimentation with Phonogel. This problem potentially seriously limits the usefulness of Phonogel as a vocal fold augmentation agent.

With overinjection of Teflon into the paralyzed fold, further shifting of the PGG peak to the right was observed. Several trials of phonation were recorded after an adequate amount of augmentation was exceeded; the ShQ value was not calculable because the peak of the PGG now was shifted beyond the plateau phase of the EGG. Figure 6E depicts the EGG and PGG waveforms from one trial of Teflon overinjection. It was observed that with overinjection of Teflon there was a reversal of the relationship between the tension in the normal and that in the paralyzed folds. The overshifting of the ShQ probably represents the flaccid fold's now being the least compliant of the two folds.

It is important to note that the two-mass vibration occurred after injection, when the stiffness or mechanical compliance of the paralyzed fold most closely approximated the mechanical compliance of the normal fold. When this occurred the subglottic air pressure was distributed evenly over the paralyzed and nonparalyzed vocal folds, and the travelling mucosal wave was resumed. It is our contention that the reappearance of the travelling mucosal wave after augmentation allows the acoustic signal to improve the amplitude in the third and fourth harmonics because of the asymmetric nonsinusoidal nature of the glottic airflow.

Future work should attempt to define a method for obtaining equivalent mechanical compliance in the paralyzed and nonparalyzed vocal folds during intraoperative Teflon injection.

It should be noted that application of our results to clinical cases may be limited because laryngeal tension was controlled by "electric" stimulation of the laryngeal nerves. However, preliminary experience with a clinical case of laryngeal paralysis treated by percutaneous injection of Teflon supports our results.

CONCLUSIONS

Photoelectric measures were useful in the evaluation and treatment of laryngeal paralyses. Both Teflon and Phonogel changed vibration of the paralyzed fold toward a more normal two-mass upper and lower margin system of vocal fold vibration. The ShQ, a measure useful in differentiating the normal versus flaccid paralysis states, returned to normal values when the mechanical compliance of the respective vocal folds was approximately equal.

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INTERNAL ANATOMY OF THE LARYNX AND THE SPREAD OF CANCER

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This project investigated the potential spread of cancer within the larynx by an analysis of the permeability and resistance of the subepithelial spaces. Permeation and diffusion of dye injections in the loose connective tissues below the epithelium was analyzed by histologic preparations in the sagittal plane. The margins of the infusion were identified grossly and microscopically, and the influence of the fibrous, elastic, and muscular components was analyzed with reference to the dispersion of the dyes. A close correlation between laboratory data from normal postmortem larynges and clinical problems of laryngeal cancer was observed in this study.

KEY WORDS — cancer, larynx.

INTRODUCTION

Conservation operations upon the larynx are proven contributions to modern laryngology, since the localized cancer may be totally extirpated and the function maintained by sparing the vocal mechanism by partial or subtotal organ resection.[1-9] The specific technical procedures are based upon an accurate assessment of the perimeter of the lesion and development of reconstructive maneuvers to restore function. The overall theme of this report is an analysis of the laryngeal components that make this type of regional surgery possible.

The specific goals of this project are 1) analysis of the spread of invasive tumor within the submucosa of the larynx, 2) identification of the structures that modify or facilitate the peripheral growth of neoplasm from a central nidus, and 3) a comparison between these anatomic data from in vitro specimens and human in vivo cancers of the larynx.

LITERATURE REVIEW

There is a large body of literature concerning the gross and microscopic anatomy of the larynx and its relationship to cancer surgery. The authors have restricted the references and related information for brevity; nonetheless, the data cited in this review are sufficiently comprehensive for the goals of this project. The previous material can be described in terms of 1) basic morbid anatomy, 2) dynamic anatomy by "in vivo" analysis of tissue planes and spaces, and 3) the progression of tumor spread documented by coronal section of the laryngeal specimen.

Basic Morbid Anatomy. The Armed Forces Institute of Pathology fascicle "Human Larynx, Coronal Section Atlas," by Tucker,[10] presents a historical review of previous investigators beginning with Spalteholz, who analyzed coronal sections of the larynx in 1903. Later investigators including Leroux-Robert[11] in 1936, Lederer[12] in 1938, Kernan[13] in 1950, Broyles[14] in 1943, and Kelemen and Sadé[15] in 1960 employed various techniques of tissue processing that identified the anatomic components within the larynx. The data published by Welsh et al[16] in 1983 described horizontal laryngeal sections after dye infusions and indicated the anatomic factors such as perichondrium, fascial planes, and ligamentous structures that modify the dispersion of the pigmented material. Other historical landmarks that warrant recognition are Morgagni's[17] (1765) and Hajek's[18] (1891) insights into the problem of laryngeal edema and suffocation, and the spaces of Pressman et al.[19] The article by the authors gives a historical review and critical evaluation of the topic of laryngeal spaces and lymphatics.[16]

Dynamic Anatomy by "In Vivo" Analysis. The compartment concept of individual submucosal spaces was reinvestigated by Pressman et al[19] in 1956, after Hajek's[18] earlier publication. This thesis suggests that there are several paired units, namely the supraglottic, subglottic, bursal, and ventricular, and the single midline epiglottic space, which limit the spread of a blue dye injected within a specific segment. From the clinical standpoint it was evident to the current authors that the pattern of metastatic behavior of malignancies was occasionally inconsistent with the edema space thesis.[20-22] Accordingly, a study was designed to identify, in live dogs, the possibility of intrinsic barriers within the submucosa. Radioactive gold (^{198}Au) in a colloidal form was instilled, and by serial coronal sections, the diffusion of the radionuclide was identified by the relative concentration of the isotope. It became apparent that during measurement of the dispersion of tracer material injected into the iso-

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Presented at the meeting of the American Laryngological Association, Palm Beach, Florida, April 23-25, 1988.

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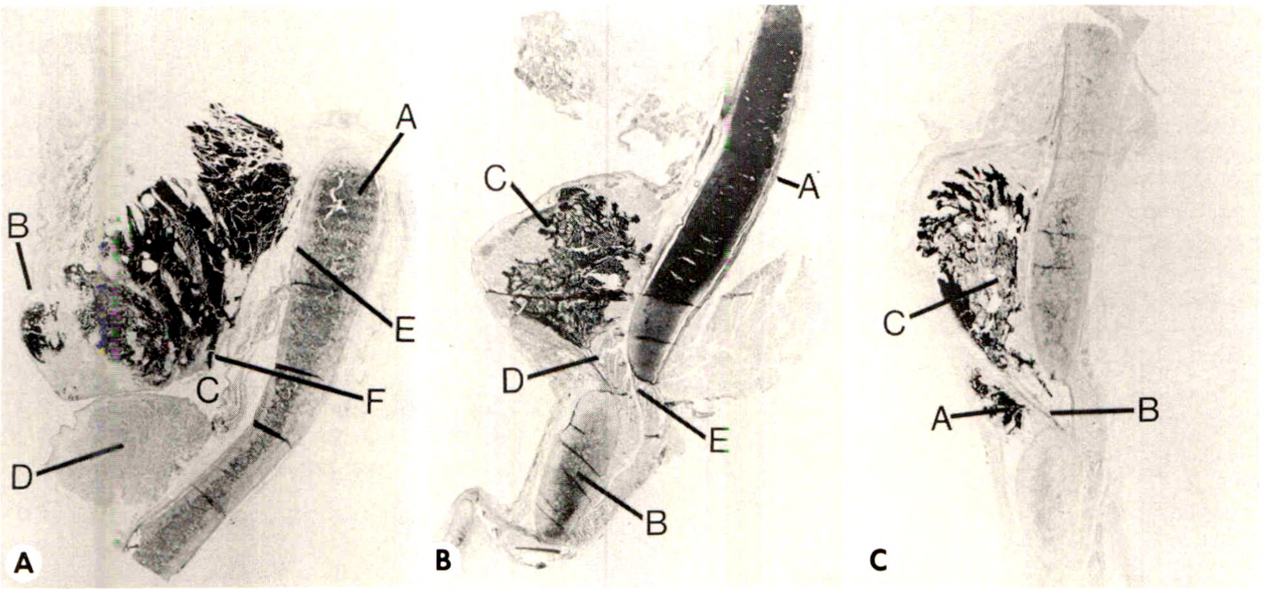

Fig 1. Microscopic analysis of subepithelial region. A) Supraglottic region. Normal structures, ie, thyroid cartilage (A), ventricular band (B), ventricle (C), and true cord (D), are identified. Dye perfusion extends to internal perichondrium (E) and inferiorly toward ventricle (F). B) Vocal cord. Framework of thyroid cartilage (A) and cricoid ring (B) is identified. Vocal cord (C) is congested with dye that appears to be contained by conus elasticus (D). Direction of gradual dispersion is toward cricothyroid membrane (E). C) Subglottic region. Site of submucosal infusion (A) is on medial aspect of conus elasticus (B). Dissection through this structure fills cordal tissue, and lateral diffusion toward internal perichondrium membrane is apparent (C).

lated supraglottic, cordal, and subglottic regions, a migration of the dye across the midline did in fact take place. The authors identified the factors of increasing mass and location with respect to the midline as the major determinants for contralateral extension beyond the artificially designated bursa.[23]

Tumor Extension by Coronal Section. Kirchner[24] analyzed the pattern of tumor growth and the potential for cartilaginous invasion of cancer of the larynx by whole section specimens. From this investigation the behavior of an epithelial malignancy was described. When we review these data by anatomic region, some generalizations can be established. The supraglottic cancers tend to behave in a less aggressive fashion than cancers in other areas, since in only one of ten specimens was the thyroid cartilage invaded by adjacent tumor.[24] In a larger series of 65 supraglottic specimens there was no evidence of invasion of the thyroid cartilage in any specimen.[25] Cordal lesions with unrestricted mobility were usually superficial to the conus elasticus (12 of 14 cases), while one showed invasion of the conus. Progression of the vocal cord lesion to limited mobility was related to further invasion of the thyroarytenoid muscle in four of six cases, while one case was due to infraglottic extension. Total fixation was associated with deep invasion of the thyroarytenoid muscle in close proximity to the thyroid cartilage (eight of 11), while three of 11 demonstrated invasion of the cartilage.[26] When the glottic lesion extended across the anterior commissure and was associated with normal or limited mobility, then the thyroid cartilage was not invaded.[26] In this clinical situation the tumor appeared to be confined within the anterior commissure tendon, the vocal ligament, and the conus elasticus. Infraglottic cancer holds a greater risk for framework invasion (two of three), with extension into the cricoid and thyroid cartilages in these cases.[25] Transglottic cancers are the most aggressive of the laryngeal problems, with 15 of 19 invading the thyroid (eight), cricoid (five), and arytenoid cartilages (five).[26] The predilection for tumor invasion into the cartilaginous framework is enhanced by the presence of ossification within these structures. The sites of tumor invasion include the lower third of the thyroid cartilage and the upper edge of the cricoid ring. Tumor activity is related to increased vascularity of bone, the presence of tumor angiogenesis factor, and cartilaginous resistance to capillary proliferation.[25] In each endolaryngeal cancer, the location, the size of the tumor, and the depth of invasion are highly relevant to the potential for cartilaginous invasion, the course of soft tissue encroachment, and the rate of metastatic activity.

Bryce et al[27] discussed the features of laryngeal anatomy that influence the spread of tumor. They stress the importance of the anterior commissure, the fibrous structure of the vocal cords, and the relationship to the perichondrium. The effect of the conus elasticus is emphasized in its relationship to the early subglottic extension of cordal carcinomas. Expansion of tumor into the preepiglottic space is described, and the increased lethality of the malignancy is clarified by invasion into the base of the tongue and the unrestricted lateral soft tissue areas. The courses of glottic and supraglottic carcinomas are defined from analysis of coronal tissue sections, and suggestions for surgical technique are firmly based upon the anatomic areas of tumor escape.

Olofsson[28] brought attention to the significance of the "silent" areas within the larynx, namely the supraglottic and subglottic regions, and observed that the tumor often is diagnosed in an advanced stage because of late onset of symptoms. Reference is made to the fact that anterior commissure lesions produced by subglottic extension may spread beyond the laryngeal framework through the cricothyroid membrane. A horizontal section at the level of the anterior commissure of the vocal cord that illustrates the potential for direct extension into the thyroid cartilage is depicted in this material. Also in this article, the ominous nature of cord fixation and the likelihood of cartilaginous invasion and extralaryngeal spread are emphasized.

METHODS AND MATERIALS

The current study employed postmortem human larynges of young individuals (ie, less than 22 years of age) to obviate the preparation problems and distortion of tissues associated with decalcification agents. Through the previous investigations on in vivo canine larynges the techniques of injection, preparation of tissues, and analysis were developed; these data were then applied to the current human study. We suggest that interested readers review the references with specific attention to the techniques that are described in detail.[11-14] In brief, however, the initial studies used radioactive gold and carbon particles as infusion materials that were injected into the submucosa of the supraglottic, cordal, and subglottic areas of the larynx. The rates and peripheral zones of permeability were measured by emission of the isotope, the margins were defined by histologic sequential section analysis, and the gross perimeter of the infusion was recorded by photographic prints. These techniques were applied to 12 postmortem human larynges in the intact state, since we had observed that division at the posterior commissure distorted the tissues and the resultant patterns of dispersion. The injection sites in each region (ie, supraglottic, cordal, and subglottic) are defined as the midplane of the hemilarynx, between the midline anteriorly, and posteriorly at the tip of the arytenoid cartilage. The infusion of dye is through a 27-gauge needle and is immediately subepithelial; the volume of infusate measures from 0.1 to 0.5 mL.

Following the injection of carbon particles (ie, India ink), the tissue was prepared by standard fixation methods and sectioned sagittally to provide a comprehensive tissue block from the epiglottic tip to the first tracheal ring. Appropriate dyes were employed to accentuate the fibrous and elastic structures of the endolarynx without dissolution of the particulate dye. The influence of volume infusion upon the periphery of dissection was recorded by sequential photography, and composite illustrations were compared to the corresponding histologic preparations.

The segment of this presentation that concerns our experience with human cancer problems uses the same techniques reported in animal studies. The tissues were processed in the sagittal plane, and identical staining materials for identification of the internal laryngeal components were similarly employed.

RESULTS

The endolaryngeal areas are divided anatomically to identify the specific tissue structures that affect dye dispersion within the subepithelial regions.

MICROSCOPIC ANALYSIS OF SUBEPITHELIAL REGION

Supraglottic Larynx. Figure 1A depicts the thyroid cartilage, ventricular band, ventricle, and true cord. The site of injection (point B) is subepithelial; subsequent diffusion in the loose connective tissue fills the paraglottic space and gradually approaches the internal perichondrium of the thyroid cartilage. Superior dissection follows the area of least resistance to the thyrohyoid membrane, while further incremental injections of dye reveal extralaryngeal extension through the aryepiglottic fold. Greater infusions extend in the horizontal direction to the internal perichondrium of the thyroid cartilage, superior to the thyroarytenoid muscle and the ventricular recess. Finally, massive supraglottic infusions parallel the internal perichondrium and dissect inferiorly along the deep aspect of the vocal cord toward the subglottic area.

Vocal Cord. The dye infusion enters the subepithelial loose connective tissue of Reinke's space and dissects within the muscle bundles (Fig 1B). As the volume of instillate increases, the direction of infusion is observed to be lateral, ie, toward the internal perichondrium. The pattern of inferior flow is modified by the conus elasticus initially; however, an increased volume of instillate overwhelms the transient influence of this fibrous structure and progresses toward the cricothyroid membrane. Finally, as a consequence of massive infusion, extralaryngeal spread appears through the cricothyroid membrane, which then invades the perilaryngeal connective tissues and strap muscles.

Subglottic Region. The injection in the subglottis (Fig 1C) rapidly permeates this region because of the scant submucosal tissue space. Perforation of the conus elasticus into the inferior cordal area promptly appears, with subsequent invasion of the thyroarytenoid muscle. While the internal perichondrium prevents direct extension of the dye into the thyroid and cricoid cartilages, the area of least resistance in this region is the cricothyroid membrane, in which extralaryngeal permeation is facili-

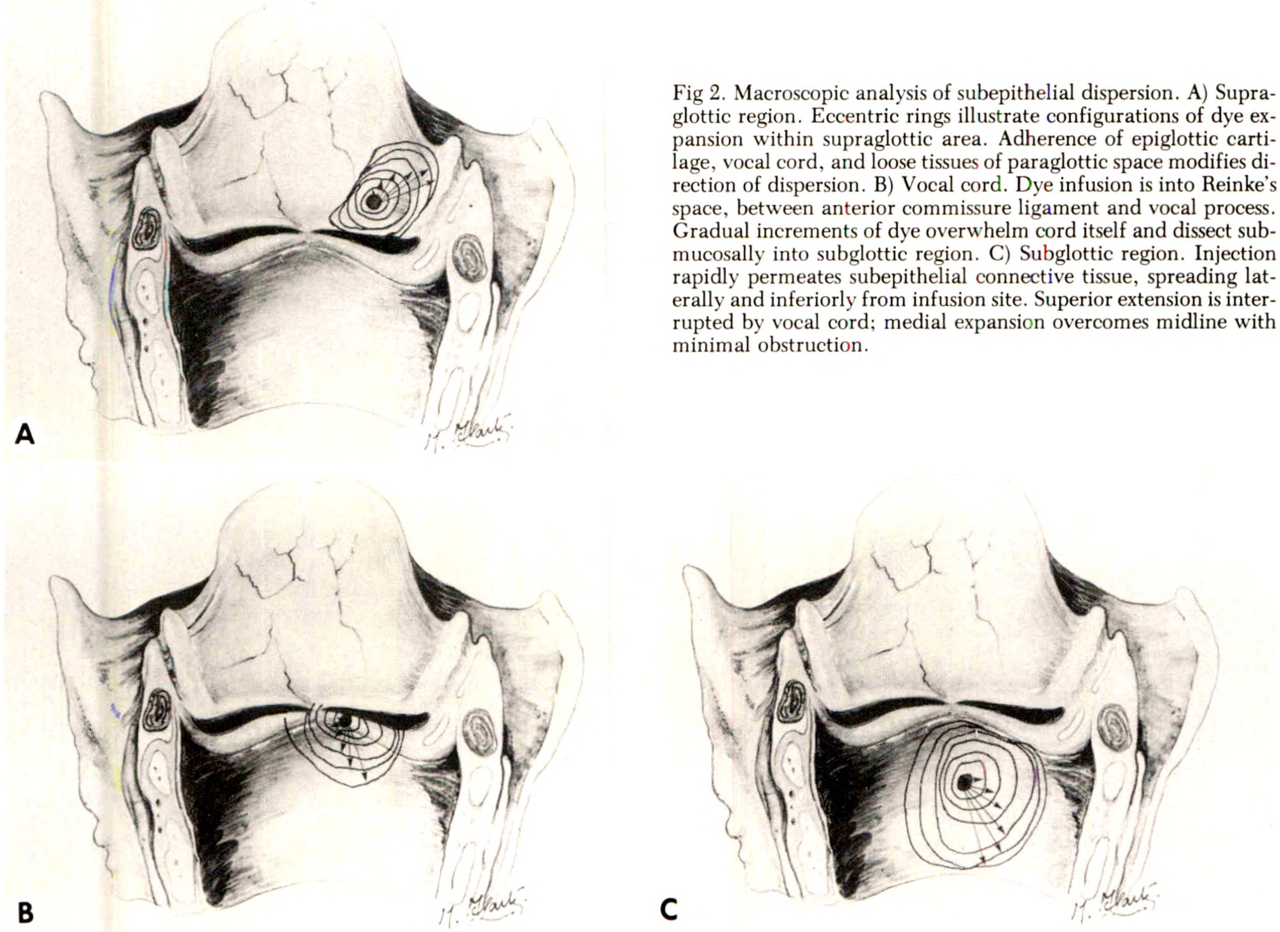

Fig 2. Macroscopic analysis of subepithelial dispersion. A) Supraglottic region. Eccentric rings illustrate configurations of dye expansion within supraglottic area. Adherence of epiglottic cartilage, vocal cord, and loose tissues of paraglottic space modifies direction of dispersion. B) Vocal cord. Dye infusion is into Reinke's space, between anterior commissure ligament and vocal process. Gradual increments of dye overwhelm cord itself and dissect submucosally into subglottic region. C) Subglottic region. Injection rapidly permeates subepithelial connective tissue, spreading laterally and inferiorly from infusion site. Superior extension is interrupted by vocal cord; medial expansion overcomes midline with minimal obstruction.

tated by the natural fibrous aperture within the cartilaginous framework. Finally, increased sequential infusions further dissect along the internal perichondrium and ultimately extend to the level of the ventricular recess.

MACROSCOPIC ANALYSIS OF SUBEPITHELIAL DISPERSION

In each of the three areas undergoing analysis the injection site is identified and the radial flow patterns of dispersion are indicated as the volumes of infusate are increased. Figure 2 is based upon photographic reconstructions of sequential infusions and reflects the influence upon the permeability of the underlying spaces by normal anatomic barriers.

Supraglottic Region. The eccentric rings representing stages of infusion indicate the resistance in the subepithelial area at the junction with the epiglottic cartilage (Fig 2A). This observation correlates with the histologic evidence presented in this report and data from previous diffusion studies that describe the firm adherence of the epithelium overlying the epiglottic cartilage and minimal subepithelial tissues. Caudally directed dispersion can be visualized deep within the ventricle, while the invading front spares the vocal cord itself, tending to dissect laterally within the loose connective tissue. Large infusions (ie, greater than 1 mL) extend beyond the aryepiglottic fold into the hypopharynx. Finally, with larger increments of dye, anterior diffusion develops below the petiole at the anterior commissure, which subsequently diffuses into the adjacent hemilarynx.

Vocal Cord. A small deposit (0.1 mL) of dye into Reinke's space rapidly fills from anterior to posterior in a horizontal plane and appears to terminate at the anterior commissure and the vocal process, respectively (Fig 2B). As the volume is supplemented, gradual pigmentation of the subcordal mucosa appears in the region inferior to the glottic rim. Additional infusions invade below the cordal substance in the subglottic region. Lastly, the appearance of anterior subglottic contralateral diffusion represents the continuity of the subepithelial spaces in this region.

Subglottic Region. The patterns of dispersion reflect some degree of internal resistance, as the eccentric flow perimeters attest (Fig 2C). The dissection from the central nidus is predominantly toward the posterior and inferior subglottic regions with gradual encroachment upon the level of the inferior cricoid ring. Thereafter, delayed migration across the midline is apparent. The area of the inferior

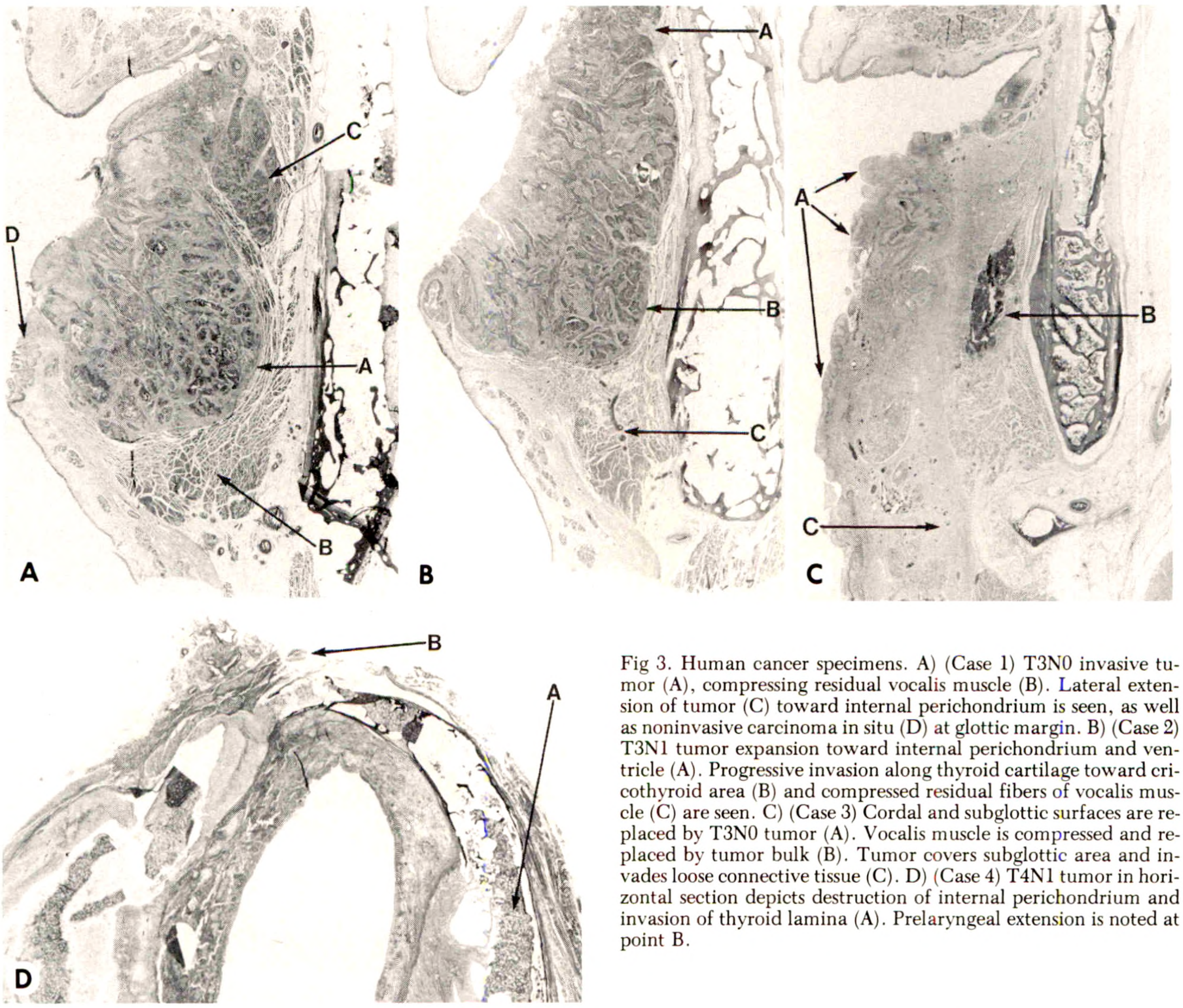

Fig 3. Human cancer specimens. A) (Case 1) T3N0 invasive tumor (A), compressing residual vocalis muscle (B). Lateral extension of tumor (C) toward internal perichondrium is seen, as well as noninvasive carcinoma in situ (D) at glottic margin. B) (Case 2) T3N1 tumor expansion toward internal perichondrium and ventricle (A). Progressive invasion along thyroid cartilage toward cricothyroid area (B) and compressed residual fibers of vocalis muscle (C) are seen. C) (Case 3) Cordal and subglottic surfaces are replaced by T3N0 tumor (A). Vocalis muscle is compressed and replaced by tumor bulk (B). Tumor covers subglottic area and invades loose connective tissue (C). D) (Case 4) T4N1 tumor in horizontal section depicts destruction of internal perichondrium and invasion of thyroid lamina (A). Prelaryngeal extension is noted at point B.

cricoid ring appears to be a relatively secure barrier limiting caudal flow. Visible extension of the dye through the cricothyroid membrane into the prelaryngeal connective tissues and fibers of the strap muscles can be appreciated with the infusion of a relatively small volume of material, suggesting minimal resistance to tumor invasion and extralaryngeal spread.

Overall, a consistent pattern of dispersion is apparent in these specimens that is contingent upon two factors: 1) the site of injection (analogous to the location of the endolaryngeal lesion) and 2) the mass of infusate (ie, the size of the lesion). Other sites of infusion beyond those described in this section, such as in the peripheral area of the endolarynx, are by location more conducive to extension into their adjacent vertical or contralateral horizontal counterpart or beyond the internal framework of the larynx. The behavioral patterns of the dye infusion in the midline, which includes the anterior commissure of the true vocal cord, the anterior subglottic area, and the base of the epiglottis, differ markedly in the regions of projection and potential for invasion from isolated unilateral lesions, since these lesions are in fact bilateral.

HUMAN CANCER CORRELATION

Sagittal and horizontal sections of selected laryngeal cancer specimens were analyzed with special attention to the pattern of neoplastic invasion and the influence of the anatomic factors described in the previous sections of this paper. We have deleted from consideration those specimens of enormous transglottic cancer in which the internal anatomy has been replaced almost totally by tumor. The impression upon tumor expansion by the conus elasticus, the quadrangular membrane, and the vocal cord ligament at this stage is negligible. Additionally, on histologic examination these fibrous components cannot be identified in the tumor bulk by the specific stains that accentuate their presence.

Case 1. Case 1 represents T3N0 glottic carcinoma with fixation of the vocal cord and irradiation failure (Fig 3A). The invasive, poorly differentiated squamous cell carcinoma (point A) appears to be invading the internal perichondrium. The residual vocal cord musculature is distorted and compressed by the tumor bulk (point B). A second area of tumor from the medial aspect of the cord in apparent, extending toward the thyroid cartilage (point C). An area of noninvasive carcinoma in situ is noted at the glottic edge (point D). The conus elasticus has been partially replaced by tumor; a marked desmoplastic response is noted at the margin of the tumor "pushing" into the muscle bundles.

Case 2. Case 2 represents T3N1 glottic carcinoma with fixation of a vocal cord and irradiation failure (Fig 3B). An invasive, moderately differentiated squamous cell carcinoma of the vocal cord extends to the internal perichondrium at the level of the ventricle (point A). The configuration of the tumor bulk suggests expansion (point B) toward the cricothyroid membrane and partial replacement of the thyroarytenoid muscle by neoplasm (point C). The conus elasticus cannot be demonstrated by selective stains in this and adjacent sections, having been replaced by tumor.

Case 3. Case 3 represents T3N0 glottic and subglottic cancer with invasion into subepithelial connective tissue and irradiation failure (Fig 3C). Grossly and microscopically, the entire cordal and subglottic surface areas are replaced by tumor (point A). Much of the vocal muscle has been destroyed by neoplasm (point B). The subglottic region contains invasive carcinoma that appears to be spreading laterally toward the conus elasticus (point C). The epithelial carcinoma is multifocal, with confluent areas in the endolarynx.

Case 4. Case 4 represents a T4N1 bulky glottic tumor with subglottic extension bilaterally (Fig 3D). This horizontal section at a level inferior to the vocal cord identifies neoplastic clumps in the ossified thyroid cartilage (point A) and within the prelaryngeal connective tissue (point B). Direct invasion and destruction of the internal perichondrium and medial plate of the thyroid lamina are evident at point A.

DISCUSSION

We emphasize the fact that this study does not apply to intraepithelial carcinomas, ie, carcinoma in situ, since the basement membrane is intact and the subepithelial connective tissues are not invaded. In the progress of invasion, the subepithelial regions including the components of connective tissue, muscle, cartilage, and perichondrium are involved and problems such as cord fixation, cartilaginous invasion, lymphatic obstruction, and extralaryngeal spread become important. This study, with its components of macroscopic mapping and tissue analysis, does indicate the regional potential for tumor spread and the relationship of malignant expansion to the fixed structures within the larynx.

A correlation between the dye studies and tumor invasion in the subepithelial tissues can be supported by analysis of the human cancer specimens. The authors have reviewed 20 larynges by sagittal section and find a close relationship to the dye studies. In the instances in which enormous endolaryngeal cancers are examined such as T3-4 transglottic or bilateral paraglottic neoplasms, the initial point of origin of the tumor cannot be identified, yet the mode of deeper invasion, though involving a relatively large area, does parallel the behavior of the more discrete regional neoplasms.

From these data patterns of tumor, expansion from a central epithelial focus may be suggested. The small lesion in the supraglottic region, as it invades the subepithelial tissue, expands into the paraglottic space and inferiorly toward the vocal cord. Further progression by tumor growth extends to the perichondrium from this point inferiorly deep to the ventricle, continuing into the deep cordal substance and finally into the subglottic region.

With regard to the vocal cord, a focus in the anterior third may invade the anterior commissure tendon and proceed along the parallel fibers to the internal perichondrium and, thereafter, into the cartilaginous framework.[20] A lesion of the middle third of the free cord appears to expand within the loose connective tissue until invasion of the muscular components extends toward the internal perichondrium and from this level inferiorly to the cricothyroid membrane and the cricoid cartilage. The limitation of tumor expansion by the conus elasticus may be less significant than previously considered, since in these cases of extensive cordal carcinoma the tumor evidently has expanded without receiving a major impact on growth or direction from this fibrous structure.

The situation of the subglottic tumor is markedly different from that of tumor in other areas of the larynx. Unfortunately, symptoms develop after the mass interferes with the airway or extension superiorly into a vocal cord produces hoarseness. The progression of growth is bilateral invasion at an early stage, infiltration of the cricothyroid membrane, and rapid spread of tumor into the prelaryngeal tissues. Inferior extension below the cricoid cartilage is less evident than superior invasion along the internal perichondrium and into the vocal muscle.

Through an analysis and comparison of the data derived from the in vivo tumor spread and the subepithelial areas of connective tissue permeability, a number of general concepts of staging and prognosis may be derived:

1. Invasion of the fixed skeletal structures seriously diminishes the potential for modified subtotal surgery, since tumor dissection may proceed along the internal perichondrium into contiguous areas beyond the scope of a subtotal laryngectomy.

2. The extent of the endolaryngeal surface area

of tumor may not correlate with the degree of invasion into the subepithelial spaces. The exceptions are small focal lesions (ie, T1) that include those on vocal cords with full mobility, small epiglottic exophytic cancers, and minute mucosal lesions of the ventricular band. Beyond this favorable stage, large area invasive tumors may extend into the depths of the subepithelial tissues and toward the periphery at an undetermined level.

The effects of radiotherapy interfere with clinical evaluation, particularly in the massive edema state characteristic of residual neoplasm, inflammatory chondritis, or postirradiation reaction without residual tumor. In several of our histologic specimens, radiotherapeutic failure was apparent in spite of the fact that the lesion on the epithelial surface had been totally eradicated: tumor nests were interspersed through the subepithelial region, invading muscle and internal perichondrium.

3. Invasion of the supraglottic cancer beyond the paraglottic region into the preepiglottic space and deep invasion of cordal or subglottic tumors toward or through the cricothyroid membrane are obvious deterrents to successful modified surgery.

CONCLUSIONS

Dye infusion studies within the subepithelial areas of the larynx demonstrate the relationship between the macroscopic margins of the pigment and microscopic areas of diffusion. Anatomic tissue resistance is demonstrated by the histologic evidence of the pathway of the dye dispersion.

The internal structures that appear to be the most significant in modification of the dye dispersion are 1) the supraglottis (epiglottic cartilage and the adherence of the epiglottic epithelium, internal perichondrium, and, lastly, permeability of the paraglottic tissues), 2) the vocal cord (vocal musculature, anterior commissure tendon, conus elasticus, and internal perichondrium), and 3) the subglottis (sparse subglottic space, internal perichondrium and epithelial proximity, and permeability of the cricothyroid membrane).

Comparison of the dye infusion studies with human laryngeal cancer cases of similar stage and primary location indicates a close parallel with the periphery or margin of the tumor, the sites of invasion, and the progression of tumor expansion.

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DEFINITIVE RADIOTHERAPY IN THE MANAGEMENT OF STAGE I AND II CARCINOMAS OF THE GLOTTIS

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Definitive radiotherapy for early glottic carcinoma continues to offer excellent control and the advantage of voice preservation. In the 13 years from January 1972 through December 1984, there were 187 patients diagnosed with squamous cell carcinoma of the glottis at the University of Virginia Medical Center. One hundred forty-eight patients were treated with definitive radiotherapy as the initial management. The 3-year disease-free survival for the irradiated population was 93.6% in stage I and 75.5% in stage II. Twenty patients had recurrences following radiotherapy, and 13 of 20 were successfully surgically salvaged for an overall determinate survival of 100% in stage I and 85.7% in stage II at 3 years. These statistics are comparable to those from our previous 16-year review of 147 patients from 1956 through 1971. Factors of prognostic significance were persistent hoarseness after radiotherapy, impaired cord mobility, subglottic extension, and multiple sites of involvement in stage I. We conclude that definitive radiotherapy offers excellent survival and that a majority of the small number of treatment failures can be managed with surgical salvage.

KEY WORDS — carcinoma, glottis, radiotherapy.

INTRODUCTION

There were an estimated 9,000 new cases of glottic carcinoma in 1988.[1] Fortunately, most of these lesions present early as stage I or II and are curable with radiation therapy or surgery. The radiotherapeutic approach to early laryngeal malignancy incorporates a radical treatment without sacrificing functional competence and with minimal morbidity to normal tissue. Definitive radiation therapy with surgical salvage is our treatment policy for stage I and II glottic carcinomas, since it offers excellent control and the advantage of voice preservation for the majority of patients. It is a generally accepted approach in the management of T1 lesions and is easily supported by previous studies from this institution showing a 96% 5-year adjusted survival among patients treated with radiation therapy at the University of Virginia.[2,3] Mittal et al[4] had a 97% 5-year adjusted survival with irradiation in 177 patients, and the review of Harwood et al[5] of 333 patients at Princess Margaret Hospital, Toronto, showed a 96% 3-year adjusted survival and a 95% 5-year adjusted survival.

The lack of controversy on the management of T1 lesions is in marked contrast to the diversity of opinion on the proper approach to T2 and T3 lesions of the glottis. This retrospective review of 148 patients will address treatment results for T1 and T2 lesions managed with primary radiotherapy and surgical salvage for failures. Survival, patterns of recurrence, efficacy of surgical salvage, and complications are analyzed. In addition, the prognostic significance of sex, cord mobility, quality of voice after radiotherapy, and extent of disease are also examined.

PATIENTS AND TREATMENT METHODS

Patients. This study is a retrospective analysis of 148 patients with early-stage squamous cell carcinomas of the glottis treated at the University of Virginia Medical Center between January 1972 and December 1984. During that 13-year period, 187 patients were diagnosed with stage I or II glottic cancer. Thirty-nine patients were excluded from this study because they opted to be treated elsewhere or presented with recurrent cancers.

All staging was reviewed personally by the first author according to the 1983 American Joint Committee on Cancer (AJCC) criteria.[6] There were 95 patients with stage I disease (64%) and 53 patients with stage II disease (36%). Definitive radiotherapy with surgical salvage was the treatment in all cases.

The epidemiologic data are presented in Table 1. Tobacco and alcohol were strong etiologic factors, and where the information was available, 96% (136/141) of patients smoked and 87% (92/106) had a history of alcohol consumption. Hoarseness was the presenting complaint in 97.2% of patients. Other symptoms included sore throat (8%), ear pain, cough, weight loss, and shortness of breath.

The primary site of the tumor was divided almost equally between the right and left true vocal cords

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TABLE 1. PATIENT CHARACTERISTICS

	No. of Patients	*Age Range (yr)*	*Mean Age (yr)*
Sex*			
Male	133	37-86	62.7
Female	15	47-88	62.7
Race†			
White	134		
Black	14		

*Ratio, 8.9:1.
†Ratio, 9.6:1.

at 51% and 49%, respectively. Cord mobility was impaired in 16 of the stage II patients (11%) (Table 2). Squamous cell carcinoma was the histologic diagnosis in 97.2% of lesions. The exceptions were two patients with a spindle cell variety of squamous cell — one with a poorly differentiated mixed histology lesion and one with an invasive papilloma. Thirty-eight patients (25.9%) presented with or developed a second primary (Table 3). Twenty-nine of the second primaries were in the lung or another head and neck site (76.3%).

Treatment. All patients were treated on a cobalt 60 teletherapy unit. The anterior wedge pair technique was used for the majority of stage I patients, because the dosimetry obtained closely approximated the anatomy of the larynx and the desired treatment volume (Fig 1). A plaster cast was used for immobilization of the patient during treatment. Two patients were treated with lateral parallel opposed fields, because they presented with synchronous second primaries in the head and neck area that necessitated treatment of the entire neck. By physician's choice, one patient was treated with lateral parallel opposed fields with wedges.

In stage I, the average treatment portal was 6 x 6 cm, and in 97.8%, the dose was 6,000 cGy, given in 25 fractions in 93.7% of patients and 30 fractions in the other 6.3%. Two patients received greater than 6,000 cGy: one received 6,500 cGy in 31 fractions as part of a treatment to include a synchronous oropharyngeal lesion, and the other received 6,240 cGy in 26 fractions by physician's choice.

Seventeen percent of stage II patients were treated with the stage I technique. The majority, however, received radiation to the primary and neck by a lateral parallel opposed field technique (83%). The dose was 5,000 cGy in 25 fractions to an average field size of 8 x 15 cm. This volume was then reduced, and an additional dose of 1,000 to 1,600 cGy in 200-cGy fractions was given to the primary.

TABLE 2. PATIENT DISTRIBUTION ACCORDING TO STAGE AND CORD MOBILITY

	No. of Patients			
	Mobile	*Impaired*	*Unknown*	*Total*
Stage I	95	0	0	95
Stage II	36 (68%)	16 (30%)	1 (2%)	53
Total	131 (89%)	16 (11%)	1 (<1%)	148

TABLE 3. PATIENTS WITH A SECOND PRIMARY ACCORDING TO SITE

Site	*No. of Patients*	*% of Total Patients (N = 148)*
Lung	17 + +	11.5
Head and neck	12 + + +	8.1
Esophagus	2	1.4
Basal cell carcinoma	2 + +	1.4
Bladder	2	1.4
Colon	1	0.7
Lymphoma	1	0.7
Pancreas	1	0.7
Total	38	25.9%

Each + represents a patient with synchronous second primary.

Methods of Analysis. All patients had a minimum 3-year follow-up, and therefore, 148 patients were available for survival analysis. Three-year determinate and crude survival were assessed in this population. Locoregional control at 3 years with and without salvage was also evaluated. All patients also were included in the analysis of prognostic fac-

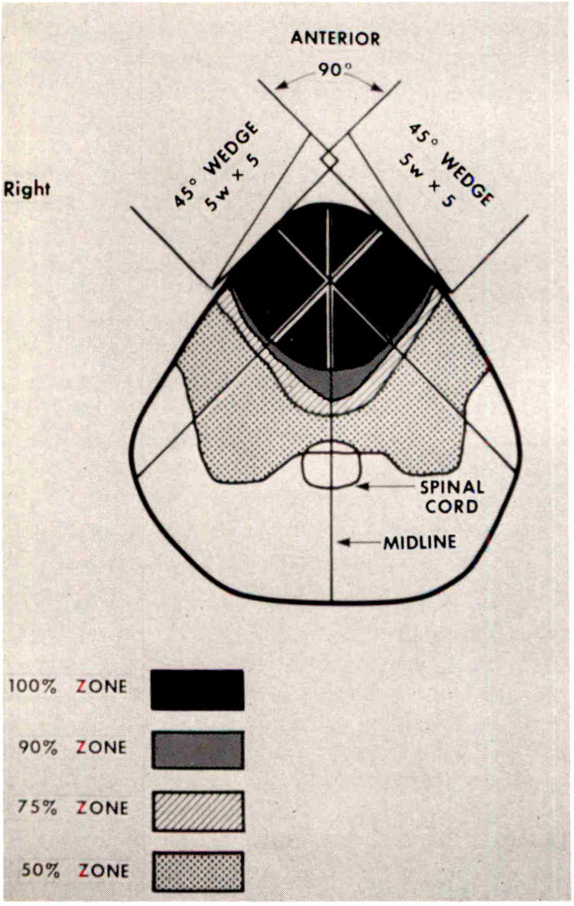

Fig 1. Dosimetry achieved using anterior wedge pair technique in stage I glottic carcinoma (field size = 5 x 5 cm, 45° wedges).

TABLE 4. THREE-YEAR SURVIVAL AND LARYNX CONSERVATION ACCORDING TO STAGE

	No. of Patients		
	Crude Survival	*Determinate Survival*	*Larynx Conservation*
Stage I	78/95 (82%)	78/78 (100%)	77/78 (99%)
Stage II	42/53 (79%)	42/49 (86%)	38/49 (77.6%)
Total	120/148 (81%)	120/127 (94.5%)	115/127 (90.6%)

TABLE 5. PATTERNS OF RECURRENCE BY STAGE FOLLOWING RADIOTHERAPY

	Primary	*Neck*	*Neck, With Dissection*	*Total*
Stage I	5/95 (5%)	1/95* (1%)	0/95 (0%)	6/95 (6%)
Stage II	12/53 (23%)	1/53 (2%)	1/53 (2%)	14/53 (26%)
Total	17/148 (11%)	2/148 (1%)	1/148 (1%)	20/148 (14%)

Interval to recurrence = 1 to 34 months.

*One patient failed outside treatment portal.

tors. Statistical significance was determined by the Fisher test.

RESULTS

Survival. One hundred forty-eight patients were analyzed with respect to 3-year crude and determinate survival (Table 4). The 3-year determinate survival was 100% and 86% for stages I and II, respectively, and 94.5% overall.

Locoregional Control. Locoregional control was achieved in 129 patients (87%) by radiation therapy alone, and in 96% of patients when including surgical salvage. The primary lesion was treated successfully by radiotherapy in 94% of patients with stage I disease and 76% of those with stage II. Patterns of recurrence by stage are listed in Table 5. The interval to recurrence was 1 to 34 months, but 90% of recurrences had occurred by 2 years.

Salvage and Laryngeal Conservation. Twenty patients (14%) failed radiation therapy, and of these, 19 were eligible for and offered salvage surgery. The non–surgical candidate received chemotherapy. The surgery performed was a partial laryngectomy, total laryngectomy, or radical neck dissection, as was appropriate.

There were six failures in stage I. Five of these were in the glottis, and one was a neck recurrence outside the treatment volume. These patients had surgical salvage, resulting in ultimate control. Four patients had a partial laryngectomy and one had a radical neck dissection. Total laryngectomy was performed in only one patient. Surgical salvage improved primary control in stage I from 94% to 100%. There was laryngeal conservation in 99% of stage I patients receiving definitive radiotherapy and surgical salvage (Table 4).

There were 14 failures in stage II, and 13 of these patients were surgical candidates. Twelve patients failed at the primary site. Of the two who had in-field neck recurrences, one also presented with distant metastatic disease. This patient was treated unsuccessfully with chemotherapy. One patient was treated successfully with partial laryngectomy. Of 11 patients receiving total laryngectomy, six achieved ultimate control. In other words, partial or total laryngectomy was successful in seven of 12 patients (58%). Radical neck dissection did not control the recurrence in the neck-only patient. Overall, the primary was controlled in 76% of stage II patients with radiation alone, and this was improved to 90% with surgical salvage. Laryngeal function and voice preservation were achieved in 77.6% of patients with stage II disease (Table 4).

ADVERSE PROGNOSTIC FACTORS

Persistent Hoarseness After Radiation Therapy. Sixteen patients (11%) remained hoarse longer than 3 months after treatment, and of these, half recurred subsequently (Table 6). Sixty-three percent of patients were salvaged successfully. This was found to be a significant prognostic factor, since 50% of patients with persistent hoarseness recurred, versus only 7% of those who regained a normal-quality voice ($p < .001$).

Impaired Cord Mobility. Cord mobility in stage II was impaired in 16 patients (30%). After treatment, 14 patients had a return to normal mobility, one showed improvement, and one with actual evidence of residual disease at the end of treatment was unchanged. Eight patients had a locoregional recurrence (50%): six in the glottis, one in the neck, and one with neck disease and lung metastases. Both neck failures were within the original treatment volume. Surgical salvage was successful in 50% of patients. Impaired cord mobility was a significant adverse prognostic factor ($p < .001$), with half of those patients presenting with impaired cord mobility recurring at either the primary or in the neck, versus 9% of those with normal cord mobility.

Patients in stage II were analyzed with respect to their pretreatment cord status and posttreatment voice quality. Voices were described as normal or

TABLE 6. PATIENT DISTRIBUTION ACCORDING TO STAGE AND QUALITY OF VOICE AFTER RADIOTHERAPY

	Voice Improved to Normal	*Voice Improved But Not Normal*	*Remained Hoarse*
Stage I (No. of patients)	71 (78%)	13 (14%)	7 (8%)
Stage II (No. of patients)	30 (62%)	9 (19%)	9 (19%)
Total*	101 (73%)	22 (16%)	16 (11%)

*Nine patients are still being evaluated.

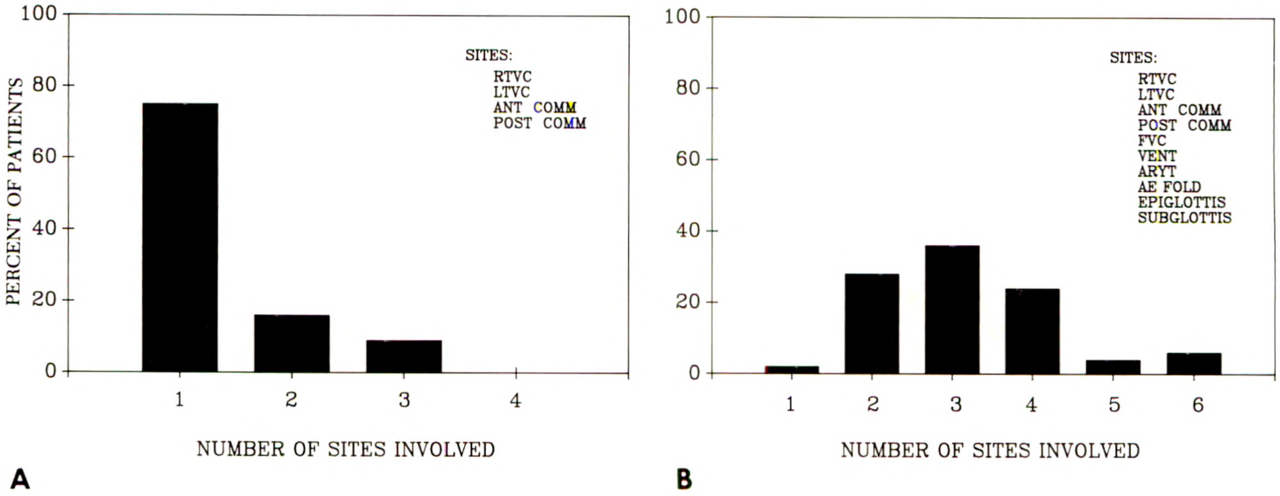

Fig 2. Patient distribution according to number of sites of involvement in A) stage I and B) stage II. RTVC — right true vocal cord, LTVC — left true cord, ant comm — anterior commissure, post comm — posterior commissure, FVC — false vocal cord, vent — ventricle, aryt — arytenoid, AE fold — aryepiglottic fold.

abnormal, with the abnormal group encompassing those patients whose voices improved but never became normal and those with hoarseness that persisted for greater than 3 months after treatment. Complete information was available in 47 stage II patients. Seventy-five percent of patients with an abnormal voice who presented with impaired cord mobility recurred. This was significant at $p = .05$ (Table 6).

Extent of Disease. Local extent of tumor was defined both in terms of gross volume as a function of number of sites in the larynx involved with disease (Fig 2), and with respect to specific sites of extension in each stage. Subglottic extension in stage I was defined as less than 5 mm. The prognostic significance of both variables was analyzed.

Bulk of disease, expressed as multiple sites of involvement, was significant in stage I. Thirty-three percent of patients with three sites of involvement recurred, versus 1% with a single site ($p < .004$). Bulk of disease was not a prognostic factor in stage II disease.

Subglottic extension was found to correlate ($p < .01$) with an adverse outcome, with 33% of patients recurring with involvement, as opposed to only 11% in its absence.

Anterior commissure involvement in stage I did not affect prognosis. Sex and histologic findings were insignificant as factors with respect to recurrence.

Quality of Voice After Radiotherapy. Patient distribution according to stage and quality of voice after radiation therapy is shown in Table 7. There was an improvement in voice quality in 89% of patients, and 73% actually returned to normal.

Complications. Complications were minimal. Chronic hoarseness was a problem in 11% of patients. Two patients (1%) developed laryngeal edema of a severity requiring permanent tracheostomy. One of these patients had had several biopsies to rule out recurrence; the results were negative, but the biopsies may have aggravated the edema.

DISCUSSION

The policy at the University of Virginia for the management of stage I and II lesions of the glottis has been consistent in the majority of patients for the last 20 years. This most recent review again emphasizes the efficacy of radiation therapy with surgical salvage as the treatment of choice. The 3-year determinate survival with this approach is 100% for stage I and 86% for stage II, or 94.5% overall.

Locoregional control was achieved in 87% of patients with radiation alone, and in 96% when surgical salvages are included. The larynx was conserved in 91% of patients overall, providing them with normal vocal communicative ability.

Six patients with stage I disease had a primary recurrence, and five accepted surgical salvage. Partial laryngectomy was the conservative approach in 80% of the patients. It was successful in each case and should be considered seriously as the salvage option of choice. Primary control was accomplished in 76% of patients with stage II disease, with 96% neck control; therefore, the major site of failure was the larynx when radiotherapy alone was used. The surgical salvage in these patients was 58% (7/12). Ultimately, therefore, primary control for stage II

TABLE 7. LOCAL RECURRENCE IN STAGE II PATIENTS WITH IMPAIRED VOICE AND/OR CORD MOBILITY

No. of Patients Recurring	*Mobile Cord*	*Impaired Cord*
With normal voice	2/24 (8%)	1/6 (17%)
With abnormal voice*	2/9 (22%)	6/8 (75%)†

*Voice improved or remained hoarse, but did not return to normal.

†Local recurrence in patients with abnormal voice and impaired cord mobility was significant ($p = .05$).

was 90%. We feel that patients with poor prognostic factors, especially impaired cord mobility combined with an abnormal voice, should be followed more closely in an effort to improve the surgical salvage rate.

Our treatment technique, dose, and fractionation are almost uniform in all patients with stage I or II disease, respectively. It has not led to an increase in complications, and voice quality improved or returned to normal in 89% of patients. In those patients surviving 3 years, the larynx was retained in 99% (77/78) of stage I patients and 91% (38/42) of stage II patients. Thus we see no reason to alter our therapeutic policy.

CONCLUSIONS

1. Radiation therapy with surgical salvage resulted in a 94% cure rate and 96% laryngeal conservation, at 3 years.
2. In stage I patients, the primary was controlled in 94% (100% with surgical salvage), the neck in 99% (100% with surgical salvage).
3. In stage II patients, the primary was controlled in 76% (90% with surgical salvage), the neck in 96% (also 96% with surgical salvage).
4. Surgical salvage was successful in 65% of patients with locoregional failure, and partial laryngectomy was successful in all stage I patients salvaged with this procedure.
5. Poor prognostic factors were persistent hoarseness after radiotherapy, impaired cord mobility prior to treatment, subglottic extension, and involvement of three sites in stage I.
6. Complications were minimal, with 11% of patients demonstrating chronic hoarseness and only 1% requiring a tracheostomy secondary to laryngeal edema.
7. There was a high incidence of second primaries (26%) and intercurrent deaths (13%) in this patient group.

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Ann Otol Rhinol Laryngol 98:1989

IMAGING CASE STUDY OF THE MONTH

ONCOCYTIC CYST OF THE LARYNX

MICHAEL TOUBBEH, MD
PATRICIA KANDALAFT, MD
ALLAN P. WOLFF, MD
CHICAGO, ILLINOIS

Oncocytic cysts of the larynx are relatively uncommon lesions and rarely cause airway obstruction. We have encountered a case of an oncocytic cyst that truly occluded the glottic inlet.

CASE REPORT

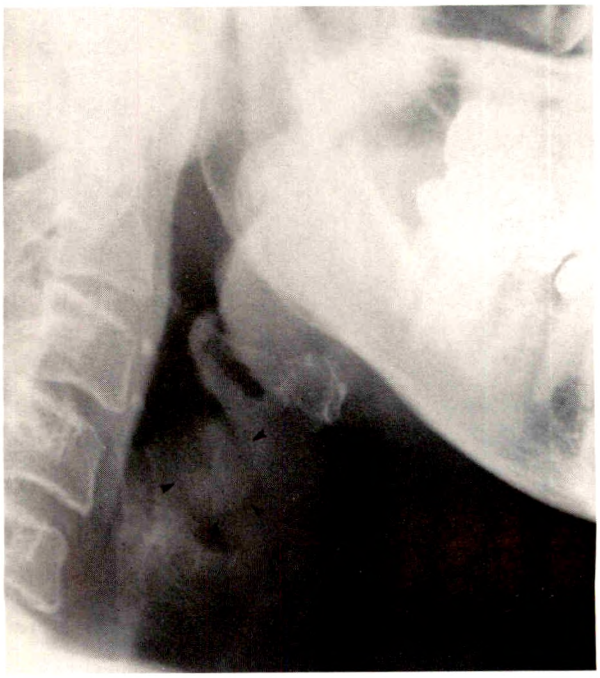
A 68-year-old woman with a 40-year smoking history presented to the emergency room with progressive upper airway obstruction and hoarseness. Attempted visualization of the larynx by the emergency room personnel was unsuccessful. A soft tissue lateral neck radiograph revealed a large marble-sized mass at the level of the glottic inlet. The attending otolaryngologist performed fiberoptic laryngoscopy, which showed a smooth cystic-appearing mass, 3 cm in diameter, almost completely occluding the glottic inlet. The patient was taken to the operating room, and with use of awake topical anesthesia, the cystic lesion was incised and allowed to drain. The airway was opened immediately.

Two weeks later, the patient underwent formal laryngoscopy. A 0.5-cm solitary mass was seen emanating from the right laryngeal ventricle, and it was removed with cup forceps. The pathologic diagnosis was oncocytoma.

DISCUSSION

Oncocytic cysts of the larynx have been referred to by a variety of names, including oncocytoma, oncocytic papillary cystadenoma, and oxyphilic granular cell adenoma.[1] They are found most commonly in the ventricles or the false vocal cords; however, all laryngeal locations have been reported. Often these lesions are multiple.

Microscopically, these cysts contain papillary projections that are lined by oncocytes, large cells with eosinophilic granular cytoplasm. Their histologic picture is reminiscent of Warthin's tumor except for the absence of a lymphoid component. Their etiology is controversial; however, they are generally considered to be a hyperplastic or metaplastic process, as opposed to a neoplastic growth.[2]



Left lateral soft tissue radiograph of larynx outlining large supraglottic oncocytic cyst (arrowheads).

Oncocytic cysts are benign, with infrequent potential for recurrence. Management is conservative and consists of local excision and, when necessary, reexcision.[3]

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From the Department of Otolaryngology–Head and Neck Surgery, Evanston Hospital and Northwestern University School of Medicine, Chicago.

Ann Otol Rhinol Laryngol 98:1989

PATHOLOGY CONSULTATION

SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY

NOUR SNEIGE, MD JOHN G. BATSAKIS, MD

HOUSTON, TEXAS

Sinus histiocytosis with massive lymphadenopathy is a lymphoreticular disorder of obscure pathogenesis with extranodal lesions and a variable clinical course. Clinical manifestations are most often in the head and neck, with 97% of patients showing cervical lymphadenopathy and a significant but lesser number having lesional infiltrates in the upper aerodigestive tracts, particularly the nasal cavity and salivary glands.

Sinus histiocytosis with massive lymphadenopathy, also known as Rosai-Dorfman disease, has prominent, if not preponderant, clinical manifestations in the head and neck.[1] The disease may be nodal and/or extranodal, although isolated extranodal disease is rare. Ninety-seven percent of the patients have cervical adenopathy, most often bilateral.[2] Any other lymph node area besides the neck may also be affected. Thirty percent of patients show evidence of extranodal disease.[2] In the latter, half of the extranodal sites are in the upper aerodigestive tracts.

The nasal cavity is the most common extranodal area of disease, followed by salivary tissue (parotid and submandibular glands), oral cavity, pharynx, tonsils, paranasal sinuses, and trachea.[1-3] Two or more of these sites are involved in over 50% of patients with extranodal disease in the head and neck.[1,2] A pathogenetic relationship is suggested by observations that two thirds of patients with sinus histiocytosis of the upper airway or oral cavity also manifest infiltrative disease in other nonlymphoid sites (skin, bone, testes, orbit, lids, abdominal viscera, and CNS).[2] The Table outlines some features of the disease in the head and neck.

Clinical signs and symptoms relate to the lymphadenopathy, which is painless and progressively enlarging, and to mucosal infiltration producing nasal obstruction and "tonsillitis." Fever, leukocytosis, increased ESR, polyclonal hypergammaglobulinemia, and possibly an elevated Epstein-Barr virus antibody titer are other accompaniments.[1,2]

The clinical course of sinus histiocytosis with massive lymphadenopathy is variable. Most patients manifest an indolent course with remissions and exacerbations. Spontaneous remission is possible, usually ensuing within the first few years after diagnosis. Persistent disease for 5 or more years occurs in one third of patients.[2] There is a mortality rate of approximately 7%, but the fatal outcome is unrelated to duration or extent of disease or to therapy.[2] There is a significant (70%) relationship between death and evidence of an immune dysfunction.[2] Eight of the 14 known fatalities have been attributed to defined immunologic abnormalities or to unusual infections.[2]

The histopathologic features of the disease are consistent regardless of nodal or extranodal involvement. The most distinctive cell is the histiocyte, a cell with abundant eosinophilic cytoplasm, bland-appearing nuclei (many with prominent nucleoli), and an irregular cell border (see Figure, A). A characteristic feature of the histiocyte is its phagocytic nature (see Figure, B). More prominent in lymph nodes, this phagocytosis includes lymphocytes, plasma cells, and inflammatory debris. Other cells in the lesional tissue are lymphocytes, plasma cells, and occasional polymorphonuclear

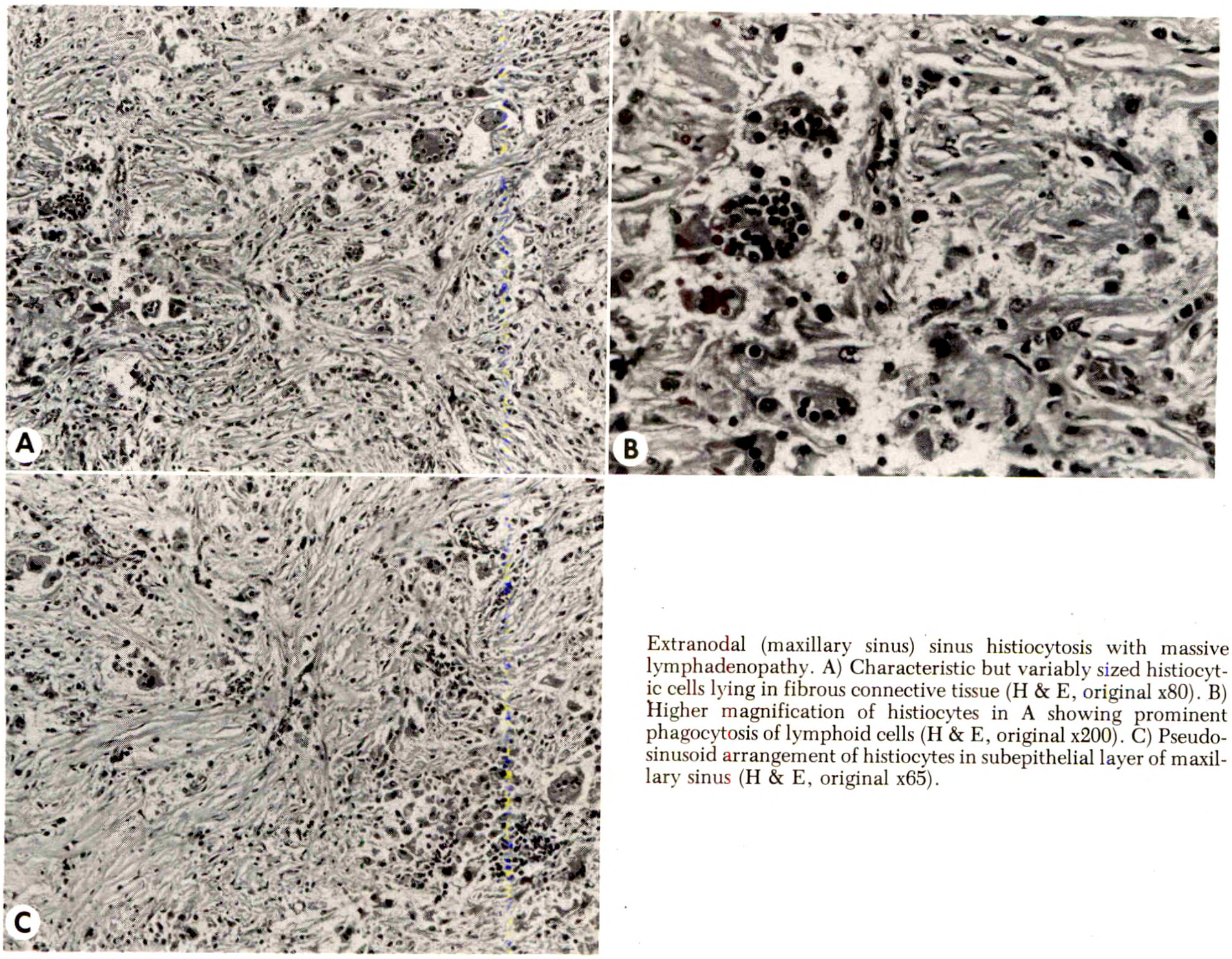
HEAD AND NECK FINDINGS IN SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY

Feature	*Findings*	*Comments*
Sex of patients; age of patients at diagnosis	M:F, 2:1; 6 mo to 67 yr	Two thirds of subjects affected within first decade; 80% before 20 yr
Lymph node involvement	97% of patients have cervical adenopathy	May be bilateral; less often solitary
Extranodal involvement	30% of all patients	
Extranodal involvement in head and neck	15% of all patients	Preponderantly in nasal cavity and/or salivary glands
Mortality	7% of all patients	No apparent relation to age, involvement, or duration; 70% have evidence of immune dysfunction

---

From the University of Texas M. D. Anderson Cancer Center, Houston.

REPRINTS — John G. Batsakis, MD, Division of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.



Extranodal (maxillary sinus) sinus histiocytosis with massive lymphadenopathy. A) Characteristic but variably sized histiocytic cells lying in fibrous connective tissue (H & E, original x80). B) Higher magnification of histiocytes in A showing prominent phagocytosis of lymphoid cells (H & E, original x200). C) Pseudosinusoid arrangement of histiocytes in subepithelial layer of maxillary sinus (H & E, original x65).

leukocytes. In lymph nodes, there is a dilation of sinuses filled with the typical histiocytic cells. In subepithelial tissues, the aggregates of histiocytes often align themselves into cords or nests surrounded by lymphoid cells, thereby creating an illusion of sinuses (see Figure, C).

Pathogenesis of sinus histiocytosis with massive lymphadenopathy is unknown, and to date, cytologic malignant transformation has not been observed.[2] The spectrum of clinicobiologic severity seen in disease, however, seems to indicate that it lies between benign reactive lymphoreticular hyperplasia and lymphoma.

Various modalities (radiation, chemotherapy, corticosteroids) have been used in palliative roles, with little or no effect on the course of the disease.[2]

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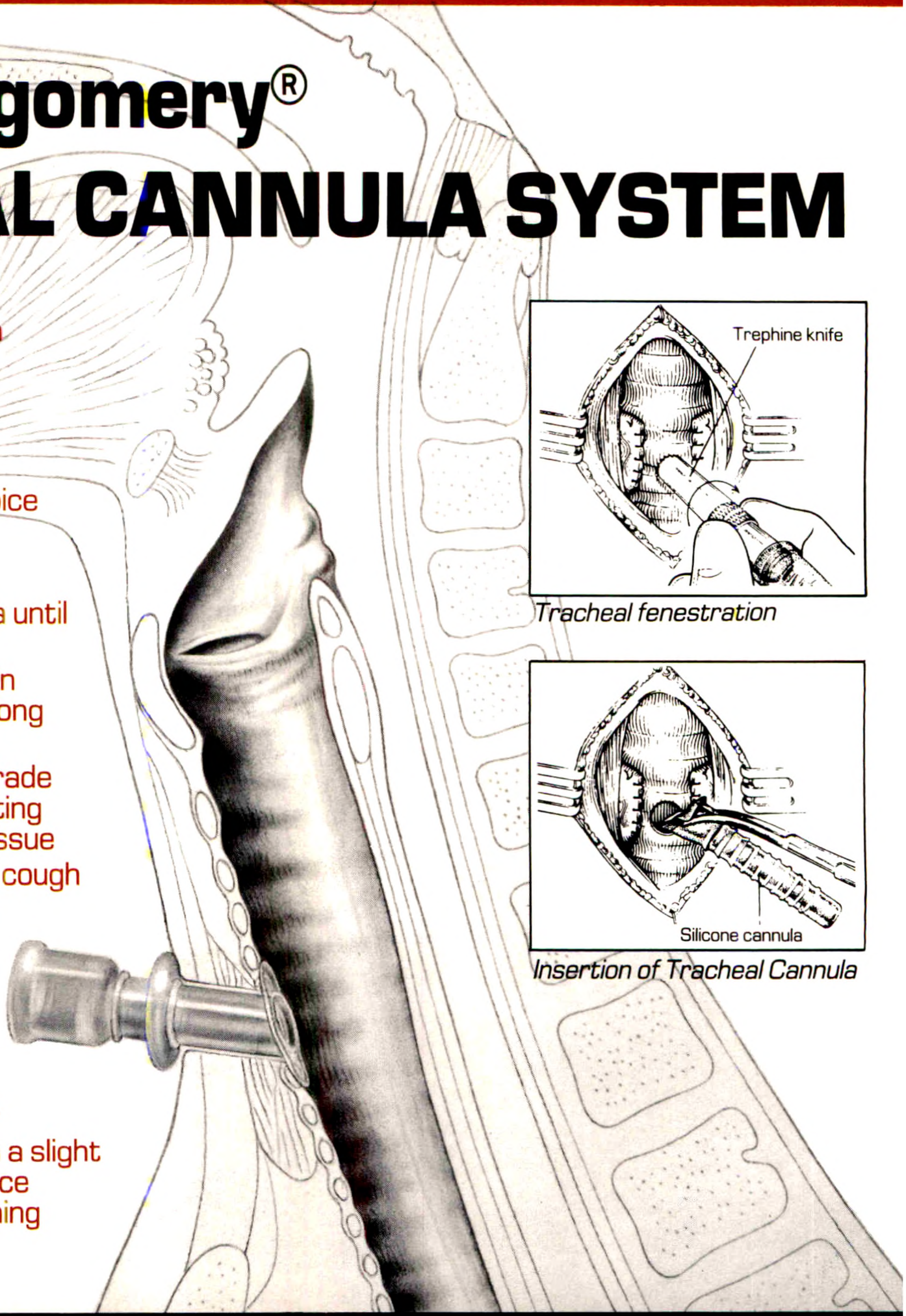

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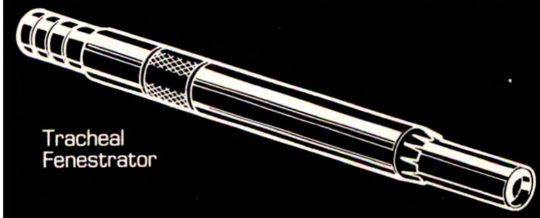

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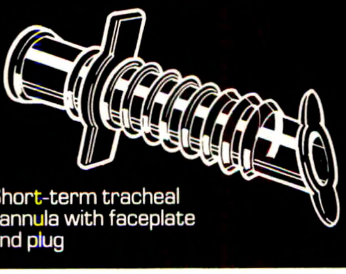

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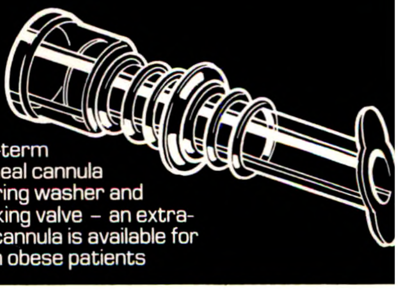

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EDITORIAL

HOW TO WRITE A SCIENTIFIC PAPER

Writing a good scientific paper is difficult. No one ever said it is easy. After having written over 150 articles I find it less arduous and painful than 30 years ago, but it is still difficult. So if you find that the next paper you write flows freely from your fingertips with painless ease, tear it up. It is probably no good.

First, why write a scientific paper? It is because of the audience you can reach with what you believe to be an important message. In presenting your data and conclusions through discussion with your colleagues you can reach dozens. At a national meeting you can reach hundreds. Only through publication in an international journal can you reach thousands.

A great advantage in publishing in a journal is that *good* journals have a process of critical review. The editor chooses two or more international experts in the specific area of your paper who meticulously go over the paper, make written detailed criticisms of the manuscript, and make one of three recommendations: accept for publication as is (5%), reject (50%), or return to author for revision with advice (45%). Any manuscript can be improved (even this Editorial, which has gone through five revisions with the help of nine critics). I have found that our very good authors appreciate the help and suggestions provided by reviewers just as much as the average author, and in some cases even more. You will find very few editors of major journals who routinely accept/reject all or a majority of manuscripts. Such an editor is not doing the job.

Now, to the matter of actually writing the article. There are a number of categories of articles, such as basic laboratory research, applied (clinical bearing) laboratory research, retrospective case analysis research, prospective clinical trials, and review articles. The following will as a rule apply to all the foregoing except the pure review article.

The article generally should be divided into seven clear sections, each with its specific purpose and containing only the material specific to it. There should be little cross-information among sections, because this leads to confusion engendered by fuzzy thinking. They are 1) Abstract, 2) Introduction, 3) Materials and Methods, 4) Results, 5) Discussion, 6) Conclusions, and 7) Summary.

1. **Abstract.** This is a single paragraph that leads the article. It states in a few sentences the *purpose* (intent) of the article, briefly *how it was carried out*, the *key findings* of the study, and the *conclusions* reached. No references are used, and no prior work is mentioned in the abstract (unless it is key to the sense of the article, eg, a refutation).

2. **Introduction.** The Introduction includes a statement as to the *reason(s)* you did the study (confusion in the field, significant differences between your data and others', an advance in technique or analysis or result, etc), *how* your results bring new light to the problem (why the reader should be interested in what you are saying), and *what* you are going to prove (demonstrate, improve upon, extend, refute, etc), and *why* it is important to do so. The work of others should be pointed out extensively in this section by references. This will indicate the work done to date so readers are aware that your work is current. References should not be exhaustive as in a master's thesis or doctoral dissertation; only key references should be noted and number usually one or two dozen.

3. **Materials and Methods.** For most authors this is the easiest place to start the writing, since it is the most straightforward. It includes the population analyzed and the reasons for it, standardization of the population for research and statistical purposes, the way(s) the population was analyzed, and the justification for doing so. The population data naturally appear

here. Data units are defined appropriately and referenced. In research articles it is important to include details that enable other investigators to replicate your study (strains of animals used with age or weight, lesioning methods, methods and instrumentation used in data collection, etc). The *outcome* data (results) can appear here, or in the Results section, depending upon the type of paper and the flow of the argument, but generally as a separate section.

4. **Results.** This section is, of course, the heart of the article. It is the reason for seeking publication. It thus must be able totally to withstand scientific scrutiny, and not simply be descriptive like the Materials and Methods section. The "n" must be significant; exclusions, if any, justified and cited; the controls adequately set up and sound; and p values distinguishing them significant. Appropriate tables must be set up, and by that I mean those necessary to document a point made in the text. Those points adequately made in the text need no tables. Here will appear experimental illustrations documenting findings, with findings clearly pointed out by arrows or labels, and crisp, concise legends.

5. **Discussion.** In the Discussion a restatement of the problem defined in the Introduction is made, the appropriateness of the experimental approach as put forth in the Materials and Methods is explained, and the hypothesis stated in the Introduction is identified as supported qualitatively and quantitatively (that is, statistically) by the results. In this section it is important in most types of papers to iterate the *significance* of the work performed, unless it is obvious. It is also permissible to speculate on the *impact* of your findings, wherever it may be appropriate.

6. **Conclusions.** In this section, which is seldom more than a paragraph, a statement as to what you believe you have proven or demonstrated is made. Conceptualizations and not data are appropriate to this section. Also, a part of this section is a statement as to what further work is indicated to provide further definition of the problem, and what importance there is in doing so.

7. **Summary.** This is the least necessary section of the group, and should be used only if a strong final statement seems needed, such as when the work presented strongly supports prior work in a controversial area, or clearly refutes prior work that has been accepted generally.

Now that the science of your scientific paper has been demonstrated, let us address the mechanics.

Each journal has a specific format in which manuscripts must be submitted. It varies somewhat from one journal to another, but the manuscript will not be accepted for review unless all the requirements are met. To find these, pick up an issue of the journal to which you wish to submit a manuscript and look for the page entitled "Instructions to Authors." This page may be printed in alternate issues, so look in another if it is not in the one at hand. Follow these instructions to the letter.

If you are not fluent in idiomatic English and you submit to a journal published in English, have your manuscript read and corrected by a physician or basic scientist who is fluent in the language. An editor may decide that a manuscript is not ready to be sent out for review because the English is such that the meaning of the author is not clear.

Finally, a word about your response to your critics (critic: Greek *krites*, a judge, discerner). Reviewers are, first, judges (yes/no), and second, helpers. My experience is that a new manuscript has a greater chance of acquiring the latter role than the former. That means your article will be accepted if appropriately revised. Your response to advice for revision should be to consider each point raised very carefully, revise according to the reviewers' comments, and, in response to points with which you cannot agree, defend adequately the position you took (the reviewer may then agree with you). Ignoring criticism almost always will result in further delay (rereview) or outright rejection.

Godspeed, and remember that our journals are not only for academicians but for all those who have a message.

Brian F. McCabe, MD, Editor

Ann Otol Rhinol Laryngol 98:1989

HEARING IN CHRONIC SUPPURATIVE OTITIS MEDIA

GEORGE G. BROWNING, MD, FRCS STUART GATEHOUSE, PHD

GLASGOW, SCOTLAND

In individuals with chronic otitis media, mixed hearing impairments are common but it is unclear whether the raised bone conduction thresholds are a reflection of the pathologic process affecting the inner ear or a combination of the high prevalence of sensorineural hearing impairments in the population along with the artificial elevation of bone conduction thresholds associated with a conductive defect. A total of 395 ears with chronic otitis media but without cholesteatoma were studied. In them, once the artificial elevation (Carhart effect) of the bone conduction thresholds, which occurs whenever there is an abnormality of the sound conduction mechanism, had been taken into account, there was no difference in the bone conduction thresholds compared with those in 920 control ears. In addition, in 100 instances, the contralateral ear was normal and there was no difference in the bone conduction thresholds between the diseased and the normal ears. Raised bone conduction thresholds in chronic otitis media are considerably likely to reflect both the Carhart effect and the high prevalence of sensorineural impairments, rather than disease damage to the inner ear.

KEY WORDS — bone conduction threshold, Carhart effect, hearing, otitis media.

INTRODUCTION

There is little doubt that in some patients with active chronic otitis media the inflammatory process can extend into the inner ear and cause labyrinthitis. In them it is reasonable to postulate that there could be an associated sensorineural hearing impairment. Fortunately, labyrinthitis is relatively rare. What is less certain is the cause of the raised bone conduction thresholds that are frequently seen in individuals with chronic otitis media who have no symptoms of labyrinthitis. The main possibilities are that they are due to the inner ear's being affected by the disease or that they reflect the high prevalence of sensorineural hearing impairments within the population.[1,2] The scientific way to answer this question is to compare the bone conduction thresholds in ears with chronic otitis media with those in appropriate control ears. Some such studies[3-5] suggest that sensorineural hearing impairments are more common in chronic otitis media. If true, it would support the concept of inner ear damage, but others[6,7] suggest that this is not the case.

All these studies have used retrospective audiometric thresholds, which by their very nature are likely to result in less reliable data than those available from a prospective study in which the stricter requirements of research are built into the design, ensuring that masking is carried out to rigorous standards. This is particularly important in the measurement of reliable bone conduction thresholds, which are the prime object of comparison between those who have, and those who do not have, chronic otitis media.

In addition, many of the previous studies have failed to recognize the fact that the bone conduction thresholds are not just a measure of inner ear function. When sound is applied to the skull its transmission is not solely via the bone to the cochlea. There is an almost equal element that is transmitted via the external auditory canal and the ossicular chain to the inner ear.[8] In individuals with a conductive impairment this second mode of transmission is not so prominent and the bone conduction thresholds are not a measure of the same thing as in normal individuals or in those with a sensorineural impairment. This is a well-recognized phenomenon and an accepted problem associated with interpretation of bone conduction thresholds.[9] Though initially discussed by Carhart,[10] who noted a postoperative improvement in bone conduction thresholds following successful surgical treatment of otosclerosis, it is well recognized to occur in other disorders that affect the conductive mechanism. It has been described during fluctuations of otitis media with effusion,[9,11,12] in chronic otitis media,[6,13-16] and when an artificial conductive impairment is created experimentally by 1) air pressure changes in the external auditory canal[12,17-21]; 2) loading of the tympanic membrane[19-21]; and 3) occlusion of the external auditory canal.[12,21-26]

No official name has been ascribed to this undoubtedly common but complicated phenomenon, so for simplicity we have chosen to call it the Carhart effect. Its practical implications are considerable for the clinician.[9] In a conductive impairment the bone conduction thresholds underestimate the "cochlear reserve" and they have to be math-

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TABLE 1. BIOGRAPHIC DATA FROM 286 PATIENTS STUDIED

	Sample Size		*Sex (%)*		*Age (yr)*		*Socioeconomic Group (%)*	
							Occupation	
State of Ear	*No. Ears*	*%*	*M*	*F*	*Mean*	*SD*	*Manual*	*Non-manual*
Chronic otitis media								
Active	306	52	53	47	50	16	86	14
Inactive	89	15	48	52	48	16	78	22
Normal	100	20	45	55	45	17	74	26

ematically corrected for this Carhart effect by taking an estimate of the external and middle ear components at each frequency and subtracting this from each of the bone conduction thresholds.[26]

The aim of the present study was to assess prospectively the inner ear hearing function in patients with chronic otitis media by means of pure tone audiometry carried out in a standardized manner and using strict rules of masking. These thresholds then could be corrected for the Carhart effect and compared with the thresholds in two control groups of ears. The first control was the contralateral ear if it did not have chronic otitis media. The second control group was made up of ears that did not have a conduction defect taken from a sample of the general population controlled for age, sex, and socioeconomic group.

METHOD

A total of 286 adult patients attending the Chronic Otitis Media Clinic at Glasgow Royal Infirmary for medical management of active mucosal chronic otitis media were studied. None of these patients had a cholesteatoma, because it is generally considered that for cholesteatoma surgical as opposed to medical management is the preferred option. At this clinic all ears were examined with an operating microscope not only to enable satisfactory aural toilet but to categorize them as having an essentially normal tympanic membrane, having an abnormal but intact tympanic membrane, or having a permanent tympanic membrane defect. Ears that were otoscopically normal were used as one category of control ears. Those that had an intact but abnormal tympanic membrane because of, for example, gross tympanosclerosis were excluded from further analysis, as they could be considered neither normal nor to have chronic otitis media. Those with a permanent defect were considered to have chronic otitis media, two varieties of which were defined.

Active Mucosal Otitis Media. This diagnosis was ascribed when there was a permanent defect of the pars tensa associated with inflammation and edema of the middle ear mucosa and a mucoid or mucopurulent discharge in the middle ear or external auditory canal.

Inactive Chronic Otitis Media. This diagnosis was ascribed when there was a permanent defect in the pars tensa but the middle ear mucosa was not inflamed and there was no pooling of secretions in the middle ear or external auditory canal.

A full clinical history was taken of the presence or absence of otologic (peripheral) vertigo, previous ear operations, and previous severe head injury. Whether a patient had instilled ear drops at any time was noted; it was assumed that these might have contained potentially ototoxic drugs, and the data were separated in the analysis. A noise exposure history was taken of industrial, social, and gunfire exposure. Individuals were classified as either having no material exposure or having been exposed to the equivalent of at least 80 dBA for 40 years, 8 hours a day. The patient's socioeconomic group was classified according to either manual or nonmanual labor.

Each patient had his or her pure tone thresholds measured by one of two technicians in a sound-reduced booth by use of the British Society of Audiology's recommended procedures.[27] The air conduction thresholds were measured at 0.25, 0.5, 1, 2, 4, and 8 kHz and the bone conduction thresholds at 0.5, 1, and 2 kHz. Bone conduction measurements at 8 kHz are not standardized and at 4 kHz cannot be relied upon without occlusion of the ear canal.[28,29] This drawback can be partially overcome by inspection of the air conduction thresholds for excessive threshold shifts at 4 kHz and above. The bone conduction thresholds were invariably assessed with masking of the nontest ear by a shadow masking procedure.[30]

The hearing thresholds in the 395 ears with chronic otitis media from the 286 patients were compared with the thresholds in two control groups of ears. The first comparison, in a subgroup of 100 individuals with unilateral disease, was between diseased ear and normal ear. Here the comparison was between the air and bone conduction thresholds at each frequency. The second comparison was between the total of 395 ears with chronic otitis media and 920 ears from 460 individuals participating in the Medical Research Council's National Study of Hearing.[31] These individuals had no history of vertigo, noise exposure (as defined above), previous ear operations, ear drops, or severe head injury. They had no otoscopic evidence of ear disease and the air-bone gap on pure tone audiometry was less than 10 dB. These controls were selected for

TABLE 4. PERCENTAGE OF VARIANCE IN AIR CONDUCTION THRESHOLDS, CORRECTED FOR DEGREES OF FREEDOM

	0.25 kHz	*0.5 kHz*	*1 kHz*	*2 kHz*	*4 kHz*	*8 kHz*
% Variance explained by age, sex, and socioeconomic group	17	18	19	25	35	42
% Additional variance explained by disease	42	38	34	29	23	15
p Level associated with change in variance	<.001	<.001	<.001	<.001	<.001	<.001
Difference (dB) between chronic otitis media and control groups for age, sex, and socioeconomic group	42	39	40	40	40	37

variables age, sex, and socioeconomic group, and to use a nonparametric test on these residuals to check for any difference in their distribution. If this procedure is adopted and the Kolmogorov-Smirnov test for two independent samples is used, then no differences in the tails of the distribution of the bone conduction thresholds can be detected between the two groups once the Carhart correction factors within the ranges given above are employed.

A similar analysis was performed on the data subset of 113 ears with vertigo but no drops, noise, or operations, thus allowing vertigo to be investigated as a potential indicator of cochlear damage from labyrinthitis. Again, the differences between the disease and control groups attributed to chronic otitis media after control of age, sex, and social class fell within the published range of values for the Carhart effect.

Finally, an analysis of covariance was conducted to evaluate whether the patients with vertigo, noise exposure, previous ear operations, and potentially ototoxic drops had poorer bone conduction thresholds than the patients without these factors. No statistically significant differences were found.

DISCUSSION

This is the first prospective study that has attempted to answer the question of whether the sensorineural (bone conduction) thresholds are elevated more frequently in individuals with chronic otitis media, without cholesteatoma, than they are in the normal population. It would appear from our analyses that this is unlikely.

In the 100 individuals with unilateral chronic otitis media, the bone conduction thresholds, even when uncorrected for the Carhart effect, were similar to those in the contralateral ear. It could be argued that the contralateral ear, even though otoscopically normal and having no air-bone gap, is likely to have been affected at some time in the past by otitis media. This could be the case, but if the middle ear was hardly damaged, the inner ear is also unlikely to be damaged. Indeed, in this study, once they were corrected for the Carhart effect, the thresholds in the diseased ear were better than those in the contralateral ear, though not significantly so. This could be a reflection of the protective action that a conductive defect is thought to have on the inner ear against noise trauma. Unfortunately, the effect of noise trauma could not be analyzed in this study, because only 11 patients with unilateral chronic otitis media had significant noise exposure.

Three other papers have analyzed patients with unilateral disease in a similar manner but using retrospective audiometric data.[5-7] Despite doubt about the validity of their data (in one study the bone conduction thresholds had been assessed in only a third of the "normal" ears), in two papers there was no significant difference in the uncorrected thresholds. In the third,[7] no statistics were quoted and the magnitude of the difference had a mean of 7 dB over 0.5, 1, and 2 kHz, which is within the range of the Carhart correction factors. The findings of these three papers along with our own are in conflict with a fourth study[2] that did not report the mean thresholds but calculated the odds ratio between ears with chronic otitis media and control ears that the bone conduction thresholds would fail certain hearing thresholds. This is an unusual and inefficient method of analyzing data when a continuous measurement variable is available. No corrections were made for the Carhart effect, and it is not possible to comment on what would occur if this were to be done, because insufficient data were presented.

In the present study it was not possible in patients with unilateral chronic otitis media to control for factors such as noise exposure, previous ear operations, and the instillation of potentially ototoxic ear drops, because the numbers were too small. It was possible to consider the presence or absence of each of these factors as covariates in the comparison of the thresholds in the total sample of 395 ears with chronic otitis media with the 920 ears taken from the population data.

TABLE 5. PERCENTAGE OF VARIANCE IN BONE CONDUCTION THRESHOLDS, CORRECTED FOR DEGREES OF FREEDOM

	0.5 kHz	*1 kHz*	*2 kHz*
% Variance explained by age, sex, and socioeconomic group	20	17	29
% Additional variance explained by disease	5	5	8
p Level associated with change in variance	<.001	<.001	<.001
Difference (dB) between chronic otitis media and control groups for age, sex, and socioeconomic group	9	11	15

Having controlled for all these factors, it would appear that though the bone conduction thresholds are poorer in individuals with chronic otitis media than in controls, the magnitude of the difference is within the published ranges of the Carhart effect, which artificially elevates the bone conduction thresholds in ears with a conductive impairment. In addition, the activity state of the ear at the time of the investigation appeared to have little influence on the bone conduction thresholds. This study was not designed to investigate ears with cholesteatoma, and this allowed us to look at a homogeneous group of active disease without cholesteatoma. This is unlike some studies[4,5,7] that looked at a heterogeneous group and did not control for different disease types. In the one study[6] that analyzed them separately, no statistical comparison was made, but the ears with cholesteatoma had better bone conduction thresholds than those without.

It is concluded from these two separate analyses that although the bone conduction thresholds are frequently elevated in chronic otitis media without cholesteatoma, this is not due in most instances to the inner ear's being affected by the disease process, but may be due to a combination of the Carhart effect and the high prevalence of sensorineural hearing impairment within the general population.

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LOW FREQUENCY OSCILLATORY VENTILATION THROUGH THE SUCTION CHANNEL OF A PEDIATRIC BRONCHOSCOPE

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To determine whether low frequency oscillatory ventilation (LFOV) may be safely applied through the suction channel of a pediatric fiberoptic bronchoscope, we devised a system using a combination of jet ventilation and constant air suction, both delivered with a single interface valve. The system was tested on an in vitro lung model and on rabbits. With tidal volumes of 12 mL, inadvertent increase in functional residual capacity (FRC) measured in the lung model was minimal. All rabbits experienced marked hypoventilation ($PaCO_2$ 62 ± 2 torr) on introduction of the bronchoscope, which promptly improved with administration of LFOV ($PaCO_2$ 41 ± 4 torr). That baseline FRC remained stable indicated that air trapping did not occur. We conclude that LFOV improves ventilation in rabbits during bronchoscopy without causing air trapping. A similar system might be applied during bronchoscopy in full-term and premature infants, thus facilitating safer and more complete visualization of their airways and preserving the possibility of obtaining samples by suction.

KEY WORDS — jet ventilation, pediatric flexible bronchoscope.

INTRODUCTION

The indications for use of the flexible bronchoscope in pediatrics have increased dramatically since the introduction, 8 years ago, of the 3.5-mm outer diameter instrument.[1] The procedure is useful for many diagnostic and therapeutic purposes in children,[2,3] but its use in full-term and premature infants remains limited.[4] The tracheal dimensions of a full-term infant are approximately 4 x 5 mm, and the glottic area is even smaller.[5] Thus, although the bronchoscope can be passed through the glottis of even small premature infants, the added airway resistance often reduces ventilation to an unacceptable degree. This limits the total procedure time or necessitates withdrawal of the instrument every 30 seconds to allow free breathing.[4] Although new ultrathin flexible bronchoscopes may resolve some of these difficulties, the lack in most of these instruments of controlled distal angulation makes a thorough examination impossible, and none of them have a suction/instillation channel.[6,7] We describe here a new approach using low frequency oscillatory ventilation (LFOV) achieved by a combination of jet ventilation at 1 Hz and constant air suction, both administered via a single interface valve to the suction channel of the bronchoscope. This system should allow easy and relatively safe visualization of hitherto inaccessible airways in full-term and premature infants.

METHODS AND MATERIALS

A lung model was used to adjust the parameters of the system to deliver a tidal volume of approximately 12 mL at various compliance values and to identify gas trapping. We then tested the system on rabbits to assess the efficiency of ventilation during a prolonged bronchoscopic examination.

The LFOV through the suction channel was accomplished by alternating pulses of positive and negative pressure (Fig 1A). Positive pressure pulses were generated by a jet ventilator (Healthdyne Impulse ventilator, Healthdyne, Inc, Marietta, Ga). These pulses were superimposed on a continuous negative bias pressure generated by suction from a wall outlet. A suction trap was placed in-line between the interface valve and the wall outlet. These two sources were interfaced by use of a dual T-piece adapter with Luer fittings (Fig 1B). The suction line contained an adapter with a 3-mm diameter bleed hole to atmosphere that provided enough resistance to generate a constant negative pressure. The bleed hole could be occluded with the thumb to increase suction for secretion removal.

In Vitro Studies. The lung model consisted of a rigid-walled container whose internal volume could be varied between 0 and 3 L to simulate various compliance values. The container had a sealed top fitted with a pressure transducer and a piece of tubing to act as a trachea. System frequency response was flat beyond 5 Hz. Tidal volumes were derived from pressure excursions within the model. The bronchoscope (Olympus BF3C4 standard pediatric bronchoscope, Olympus Corp of America, New Hyde Park, NY) was sealed into the model trachea. The effective compliance of the apparatus was adjusted between 0.5 and 2.5 mL/cm H_2O to simulate a range of compliances, from that in a sick premature infant to that in a healthy newborn infant. The magnitude of the tidal volume was controlled primarily by the driving pressure of the jet ventilator,

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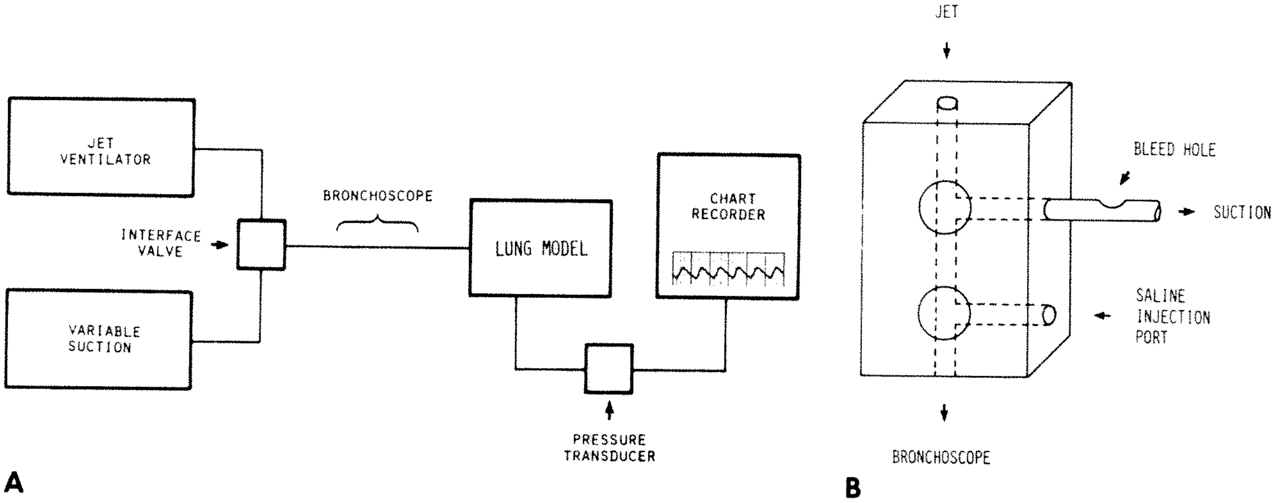

Fig 1. Low frequency oscillatory ventilation assembly scheme. A) System is attached to bronchoscope through single interface valve. Bronchoscope is sealed into lung model. B) Schematic drawing of dual T-piece interface valve.

while frequency, duty cycle, and negative bias pressure were adjusted to minimize gas trapping within the lung model. That is, elevations in the baseline pressure within the lung model would correspond to inadvertent increases in functional residual capacity (FRC) for real lungs. The jet ventilator settings used for the in vitro and animal studies were 1) driving pressure, 40 pounds per square inch gauge (psig); 2) frequency, 60 cycles/min; and 3) duty cycle, 10%.

Animal Studies. Four New Zealand white rabbits ranging in weight from 3.2 to 3.8 kg were studied. After sedation with ketamine (50 mg/kg) and xylazine (5 mg/kg) given intramuscularly, arterial and venous lines were inserted in the left ear. Maintenance doses of ketamine were given intravenously as needed. In order to mimic the tight fit of a bronchoscope in a small infant's larynx, the rabbits were intubated with a 4-mm inner diameter cuffed endotracheal tube (Mallinckrodt Inc, Argyle, NY) through which the bronchoscope was advanced. Arterial blood gases were obtained at rest and after intubation during spontaneous breathing. The bronchoscope was then advanced to a level 1 cm above the carina, and an arterial sample was obtained 2 minutes later. By 2 minutes the rabbits showed severe retractions and cyanosis because of apparent airway obstruction caused by the bronchoscope. Immediately thereafter, LFOV was begun with FiO_2 of 1.0. Arterial blood was obtained 5 minutes after the bronchoscope was positioned just above the carina and again after another 2 minutes with the bronchoscope in each of the main stem bronchi. Arterial blood gases were analyzed with a Radiometer ABL-4 blood gas analyzer (Radiometer of America, Cleveland).

Tidal volume and FRC were measured with a 20-L pressure plethysmograph as described elsewhere.[8] Briefly, the rabbit was placed in the plethysmograph with the endotracheal tube and arterial and venous lines outside the box. A complete seal was obtained. The frequency response of the plethysmograph was measured by use of a system analogous to that of Jackson and Vinegar.[9] A Validyne (MP-45-18, ± 5 cm H_2O) transducer was used to detect corresponding pressure changes within the plethysmograph. The transducer had a flat frequency response (within 5%) beyond 40 Hz. The plethysmograph itself showed a flat response (within 5%) to 27 Hz with slow roll-off up to 40 Hz. The plethysmograph was calibrated with a syringe and volumes from 10 to 30 mL in 10-mL increments. All data were recorded on a Gould recorder (model 260, Gould, Inc, Cleveland).

RESULTS

In Vitro Studies. Tidal volume delivered by the system was affected minimally by the compliance of the lung model. Volumes between 12 and 13 mL were achieved. The ventilator settings that delivered the desired tidal volume were 1) frequency, 1 Hz; 2) duty cycle, 10%; and 3) driving pressure, 40 psig. The minimal change in end expiratory pressure indicated that air trapping was negligible.

Animal Studies. Results of experiments in four rabbits are shown in Fig 2. Marked hypercapnia and hypoxemia occurred after the initial introduction of the bronchoscope. Both were promptly corrected by LFOV, even during the evaluation of the two main stem bronchi (Fig 2A). Use of LFOV decreased the $PaCO_2$ from 62 ± 2 (mean ± SEM) torr to 41 ± 4 torr, while PaO_2 increased markedly, from 28 ± 2 torr to 450 ± 42 torr. In order to correct for the differences in oxygen concentration with ventilation, we calculated the arterial-alveolar (a/A) oxygen tension ratio, which is an index of gas exchange function. This ratio is similar to the a/A oxygen gradient, but is more stable when inspired oxygen concentrations are changing.[10] The a/A ratio

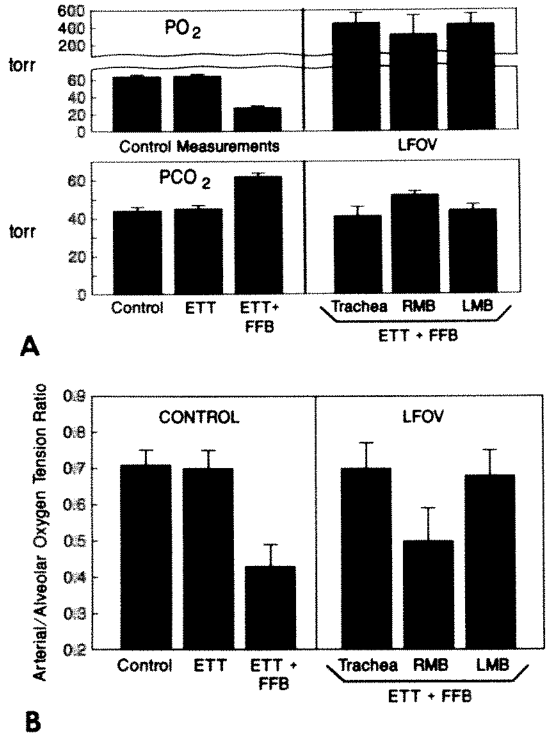

Fig 2. Results of studies on four rabbits with and without low frequency oscillatory ventilation (LFOV). Samples were obtained at rest (control), after endotracheal intubation (ETT), and after introducing flexible fiberoptic bronchoscope (ETT + FFB). LFOV was started with $FiO_2 = 1.0$, and samples were obtained while bronchoscope was in trachea, right main stem bronchus (RMB), and left main stem bronchus (LMB), as described in Methods and Materials. A) Arterial blood gas data (mean ± SEM). Hypoxemia and hypercapnia observed during bronchoscopy (ETT + FFB) did not occur when LFOV was applied. B) Arterial-alveolar oxygen tension (a/A) ratio (mean ± SEM). There is marked improvement in a/A ratio with LFOV. Decreased a/A ratio when bronchoscope is in RMB probably is due to obstruction of right upper lobe bronchus.

markedly improved while the rabbits were on LFOV (Fig 2B): from 0.4±0.06 to 0.7±0.07. Ventilation and oxygenation, as measured by the $PaCO_2$ and the a/A ratio, were worse when the bronchoscope was in the right main stem bronchus than when it was in the left.

To determine if there was any air trapping caused by the LFOV, we measured FRC during ventilation. There was no increase in FRC and thus no air trapping during use of the LFOV system (Fig 3).

On several occasions, the airways were successfully lavaged by means of the following procedure. The stopcock comprising the saline injection port (Fig 1B) was turned so that jet gases were directed out the suction port and saline from a syringe could be injected down the suction channel of the bronchoscope. The stopcock was opened to the jet and suction, and the bleed hole was immediately occluded until the airways were cleared of mucus and saline. When the bleed hole was uncovered, ventilation resumed as before. In the event that some liquid remained in the suction channel and ventilation was reduced, the interface valve was temporarily disconnected and suction applied directly to the suction channel of the bronchoscope. Our ability to ventilate and aspirate the airways resulted in continuous placement of the bronchoscope in the trachea for up to 40 minutes.

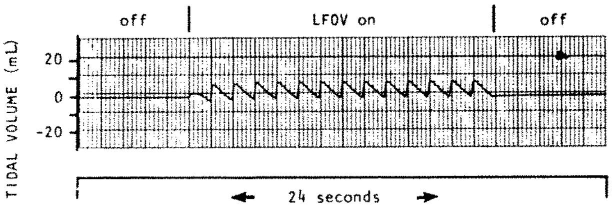

Fig 3. Pressure recording from plethysmograph in one rabbit. Absence of changes in end expiratory pressure recordings in plethysmograph indicates that functional residual capacity did not change with LFOV.

DISCUSSION

In many clinical situations, it is important to examine the lower airways of infants, neonates, and even premature infants.[11-15] Even in adults (whose airways are considerably larger), use of the flexible fiberoptic bronchoscope is associated with a decrease in arterial oxygen tension, caused in large part by the increased airway resistance produced by the presence of the instrument in the airway.[16,17] In infants, with smaller airways, this problem is worse.[1,4] As a result, the duration of the procedure is limited and the bronchoscopist is forced to withdraw the instrument every 30 seconds to allow the infant to breathe. Repeated passage of the bronchoscope through the larynx can cause laryngospasm, bradycardia, and dangerous subglottic edema. Ultrathin bronchoscopes have expanded our capabilities for airway evaluation of infants and neonates,[6,14,18] but few such instruments have a dirigible tip, and the lack of a suction/instillation port makes it impossible to instill topical anesthetics onto the larynx, remove secretions, or obtain specimens.

Our technique, consisting of a combination of jet ventilation and constant air suction, is an important step toward resolving these problems. Our initial results demonstrate that this system can deliver adequate tidal volumes that vary slightly depending on the lung compliance, without substantial progressive air trapping in the test lung. Furthermore, when the tight fit that presumably exists between the bronchoscope and the larynx in small infants was mimicked experimentally in rabbits, we were able to achieve good air exchange without increasing FRC. The LFOV in either the right or the left main stem bronchus was superior to spontaneous ventilation. Ventilation of the left lung was superior to that of the right, probably because of the inefficient right upper lobe ventilation when the bronchoscope is positioned in the right main stem bronchus of the rabbit.

Our system is completely different from those described by Monden et al,[19] Dalens et al,[20] and Labbe et al.[21] These systems required passive expiration through the space between the bronchoscope and the larynx or endotracheal tube, and the tight seal that exists in small premature infants and newborn infants was not mimicked.

During LFOV, the positive pressure pulses are directed partly down the internal channel of the bronchoscope and partly through the suction device, causing net flow of fresh gas into the lungs. During the off periods of the jet pulses, the negative bias pressure facilitated exhalation through the relatively high resistance of the bronchoscope. The frequency, driving pressure, and duty cycle of the jet ventilator, as well as the level of negative bias pressure, must be adjusted to achieve adequate tidal volume delivery without alveolar gas trapping. However, the configuration of the LFOV system makes it essentially a time-cycled, pressure-limited device that generates a rectangular pressure waveform at the opening of the bronchoscope suction channel. Thus, the tidal volume delivered to the lungs and the magnitude of gas trapping are functions of not only the pressure waveform but also the compliance of the respiratory system and the combined resistance of the bronchoscope channel and the airways. The resistance of the system is dominated by the resistance of the suction channel of the bronchoscope, so that the small additional resistance of the respiratory system was negligible by comparison and was excluded from the model during the in vitro experiments.

As a system of mechanical ventilation, LFOV is limited by the amount of feedback the clinician has regarding the adequacy of the delivered tidal volume. There is no convenient way to measure airway pressure, and esophageal pressure measurements would provide a rather insensitive index of volume delivery because of intervening lung impedance and the technical difficulties associated with the available measurement techniques. Furthermore, the variable amount of space between the bronchoscope and the larynx markedly affects volume delivery. For these reasons, the in vitro studies were designed to provide optimum ventilatory support under worst-case conditions (ie, a complete occlusion of the larynx by the bronchoscope). It is under these conditions that the patient is at highest risk of underventilation, overventilation, or barotrauma due to abrupt changes in FRC (eg, if the suction line were to become disconnected and the lungs were overinflated). When the bronchoscope does not completely occlude the larynx, the risk of overexpanding the lungs is virtually eliminated and the patient is able to modulate both the level of ventilation and FRC. In either case, clinical observation of chest rise and breath sounds provides sufficient information to assess the adequacy of ventilatory support. It should be remarked that most bronchoscopic procedures are relatively short in duration and the main concern is hypoxia rather than hypercapnia, which is easily eliminated by using an appropriate FiO_2.

We suggest that LFOV applied to the suction port of a small flexible bronchoscope may allow relatively safe and prolonged procedures in very small infants, while preserving our ability to clear the airway or obtain samples by suctioning. Of course, despite the apparent safety of this technique, these infants should have continuous cardiac and pulse oximetry measurements during the procedure. Because the technique avoids the trauma of repeated removal and reinsertion, it could prove to be an important advance in use of the flexible bronchoscope in small infants.

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XX INTERNATIONAL CONGRESS OF AUDIOLOGY

The XX International Congress of Audiology will be held October 14-18, 1990, in Tenerife, Canary Islands, Spain. For further information, contact Dr Jose Barajas, Presidente, C/Perez de Rozas, 8, 38004 Santa Cruz de Tenerife, Canary Islands, Spain; telephone (22) 27.54.88; telex 91106.

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CHEMICALLY INDUCED ESTHESIONEUROEPITHELIOMA: ULTRASTRUCTURAL FINDINGS

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Tumors of the olfactory epithelium of rats were induced with two different nitrosamines: 2,6-dimethylnitrosomorpholine and *N*-nitrosopiperidine. Both carcinogens yielded identical tumors consisting of small, undifferentiated, neuroblastic cell elements without specialized cell contact. Cell processes contained microtubuli, centrioles, and neurosecretory granules. Two kinds of rosettes were encountered frequently: neuroblastic Homer Wright rosettes consisted of undifferentiated cells, surrounding a minute lumen filled with amorphous material; and Flexner rosettes showed a higher degree of maturation. Inside their central lumen, cell processes with characteristic features of olfactory sensory cells (basal bodies, cilia, centrioles, microtubuli) could be demonstrated. The stem cell of this tumor is most likely the undifferentiated light basal cell inside the olfactory epithelium, since its ultrastructural appearance and its cytoskeleton are alike. At least under neoplastic conditions, this stem cell may likewise differentiate into epithelial cells, since transition to squamous cell carcinomas has been observed. In view of their overwhelming similarity to their human counterpart, the induced tumors are most likely to represent esthesioneuroepitheliomas.

KEY WORDS — chemical carcinogenesis, esthesioneuroepithelioma, olfactory epithelium, ultrastructure.

INTRODUCTION

Since the first description of the carcinogenic effect of dimethylnitrosamine by Barnes and Magee[1] and Magee and Barnes,[2] nitrosamines have been among the most thoroughly investigated carcinogens. A large and steadily increasing number of different carcinogens and nitrosocompounds have been investigated,[3,4] many of which showed a marked organotropic effect.[5,6] In particular, the mucosal lining of the nose often proved to be the target organ of many nitrosamines.[5,7-10]

Whereas most of these studies were performed to elucidate the mechanisms of carcinogenicity and their alterations by changing the chemistry of the carcinogens, comparatively little is known about the histology of the tumors. Only recently have some reports described the ultrastructural appearance of chemically induced tumors.[11-14] In 1965, however, Thomas[15,16] was able to show by light microscopic investigations that a high percentage of *N*-nitrosopiperidine–induced tumors in rats were of neurogenic origin. These tumors were indistinguishable from human tumors of the same region and most probably were esthesioneuroblastomas, a type of tumor that likewise was induced in hamsters by diethylnitrosamine.[17]

This observation in chemically induced tumors is remarkable for two reasons: 1) the high incidence of neurogenic tumors, since only 2% of all nasal tumors in humans are neurogenic in origin[18]; and 2) the suggested histologic specification of esthesioneuroblastoma, since this tumor is one of the rarest in humans.[19] In addition, this undifferentiated tumor is difficult to diagnose correctly,[20] so that it generally is doubted whether diagnosis of this tumor may be established on the basis of the light microscopic picture alone.[21] On the other hand, since the first electron optical investigations of this tumor by McGavran,[22] several ultrastructural features of this tumor have been reported[19,21,23,24] without finding a characteristic feature on which a safe diagnosis might be established. The most common findings were neurosecretory granules,[22,23] which are not pathognomonic in themselves. In addition, the histogenesis of this rare tumor is still a matter of controversy.[21,23]

We therefore used two different carcinogens (*N*-nitrosopiperidine; 2,6-dimethylnitrosomorpholine) that are known to induce tumors inside the nasal cavity of rats[9,15,16] in order to investigate the tumors histologically and, if possible, to elucidate their histogenesis. The tumors were investigated by different morphologic methods (light microscopy, indirect immunofluorescence microscopy for demonstration of intermediate filaments, electron microscopy, chromosome analysis), biologic procedures (cell culture, transplantation to nude mice), and biochemical investigation (peptide hormones, catecholamines). The light microscopic findings and investigations of intermediate filaments as well as the cell culture findings of the induced tumors have been described recently.[25] This report deals with ultrastructural investigations.

MATERIAL AND METHODS

A detailed description of applied carcinogens, cancerization procedure, and animal housing was given in one of our previous reports.[26] Briefly, tu-

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mor induction was initiated in 100 animals (white Wistar rats, strain Bar W ism SPF), 30 of which received *N*-nitrosopiperidine injected twice a week subcutaneously in the back. Seventy rats received 2,6-dimethylnitrosomorpholine dissolved in drinking water. In this group, 20 rats died of pneumonia within the 17th week prior to tumor growth in the others, so that 80 rats were actually studied. Since the different investigations could not be performed with the relatively small primary tumors in the rats, some of the tumors were serially transplanted to nude mice and kept alive for up to 17 passages.

Ultrastructural Technique. Immediately after an animal had been killed, pieces of tumor tissue about 1 mm in diameter were immersed in the first fixative (6 mL glutaraldehyde 25% and 6 mL paraformaldehyde 25% in 45 mL 0.2 *M* cacodylate buffer were dissolved in 100 mL distilled water, pH 7.35) and kept there for 3 hours at 4°C. After being washed in cacodylate buffer (0.1 *M*, pH 7.35) three times for 1 hour at 4°C, the tissue was postfixed for 2 hours in osmium tetroxide (a 1:1 solution of 2% OsO_4 and 0.2 *M* cacodylate buffer). Thereafter the tissue samples were dehydrated in a series of ascending alcohol solutions, 30% and 50% for 10 minutes each, then with 70% alcohol and saturated solutions of uranyl acetate for 12 hours. Thereafter final dehydration was performed with 70%, 80%, and 100% alcohol at room temperature. After immersion in the propylene oxide for 5 minutes twice, the tissue was soaked in the embedding medium, initially consisting of a mixture of propylene oxide and araldite (1:1 for 1 hour and 1:3 for 2 hours), and finally in pure araldite for 60 minutes. Final polymerization in araldite took place in gelatin capsules (0.5 mL volume) for 3 days at 60°C.[27] The gelatin capsules were trimmed (TM 60, Reichert and Jung) so that a trapezoid tip appeared.

Semithin sections were cut with glass knives on the ultramicrotome (Ultracut OMA 3, Reichert and Jung) and stained with toluidine blue (80°C to 90°C for 1 minute). Important areas were marked and further processed for ultrathin sectioning. Under visual enlargement the areas of minor importance were removed from the block with razor blades according to the notes on the semithin sections. Then ultrathin sections (50 to 100 nm) were made and mounted on copper grids (type 200, diameter 3.05 nm). The specimens were finally contrasted with lead citrate and studied with a Zeiss electron microscope (type EM 9). Important areas were documented on Agfa scientific film (No. 23, D 56).

RESULTS

The time course of cancerization, the macroscopic appearance of the tumor, and the light microscopic and the immunohistologic picture of the induced tumors were described in a previous report.[25] It was found that both carcinogens produced tumors in the same region (olfactory epithelium), showing identical light microscopic features and the same intermediate filament characteristics.[25] Since our electron microscopic investigations confirmed this congruence, the ultrastructural findings will be presented together. The tumor consisted of densely packed, small, undifferentiated round cells containing a large nucleus surrounded by a scanty cytoplasmic border (Fig 1A). The nucleus showed finely dispersed chromatin often containing a large nucleolus in contact with the nuclear membrane.

The cytoplasm of the tumor cells contained numerous polyribosomes and only a few mitochondria. The cell membranes of closely packed tumor cells did not express any kind of specialization such as desmosomes. Only occasionally were there electron-dense areas imitating a cell contact. At higher magnification, however, it became evident that this structure was situated between two adjacent cell membranes (Fig 1B). Other areas of the tumor were dominated by cell processes containing structures typical of this tumor, especially large numbers of microtubuli arranged in a parallel fashion (Fig 2A). In addition there were often centrioles (Fig 2A,B), which in some cell processes showed a marked accumulation (Fig 2B). Consistent features of the cell processes and — less often — of the perinuclear cytoplasm were dense-core vesicles[25] (Fig 2C-E).

Since the most reliable light microscopic feature of this tumor is its tendency to form rosettes,[25] this tumor permits a thorough investigation of the different kinds of rosettes on the ultrastructural level. Figure 3A shows an overview of a typical Homer Wright rosette. This type of rosette consists uniformly of typical undifferentiated tumor cells arranged in a circle around a small central lumen. The nuclei occupy the basal portions of the cells, whereas the supranuclear portion is elongated toward a sharply defined tip sometimes containing dense-core vesicles (Fig 3B). The tiny central lumen of the rosette is filled with amorphous material (Fig 3B) and a few microvilli, some of which seem to contain microtubuli. Close to the lumen, the cell membranes of adjacent cell tips are connected by tight junctions (Fig 3B).

More often these tumors expressed the other type of rosette, the Flexner rosette, which is shown in Fig 4A. Here the tumor cells surround a much wider central lumen. The light microscopically discernible internal limiting membrane[25] is ultrastructurally represented by tight junctions connecting the apical cell portions. Often there were neurosecretory granules (Fig 4D) and centrioles in various stages of differentiation into basal bodies (Fig 4B). These basal bodies were able to form cilia (Fig 4C). In addition there were microtubuli in cells

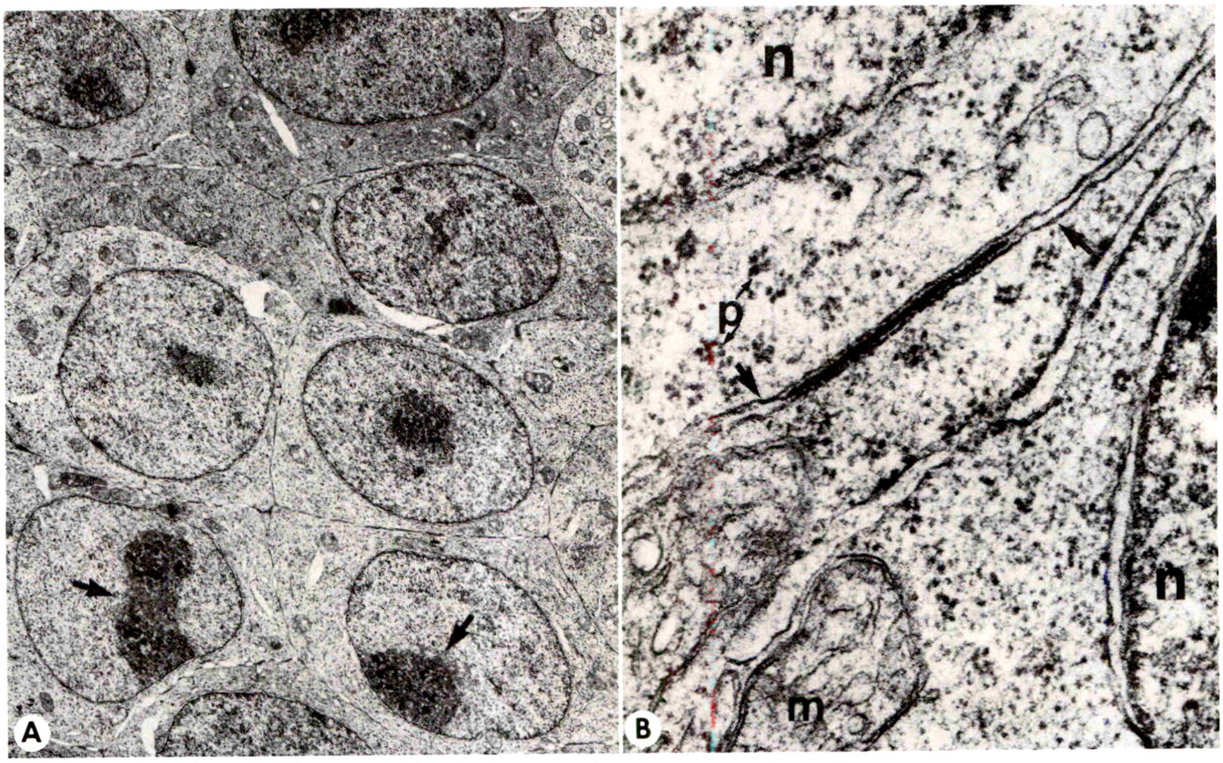

Fig 1. Undifferentiated tumor cells. A) Low magnification (original x7,200). Cytoplasm contains some mitochondria and many polyribosomes. Marginated nucleoli are seen at caudal border (arrows). Between adjacent cells, electron-dense material imitating contact structures is seen. B) Higher magnification of closely packed tumor cells (original x50,000). Electron-dense material imitating specialized cell contact lies clearly between cell membranes (arrows) of two adjacent cells. n — nucleus, m — mitochondria, p — polyribosome.

and cell processes of these Flexner rosettes. Even more typical and characteristic of this kind of tumor were ultrastructural findings in distended Flexner rosettes imitating glandular structures (Fig 5A). Here we saw bundles of nerve fibers running in irregular fashion close to the surface. At higher magnification further specializations were discernible: besides numerous microvilli there were many bulblike cell processes protruding into the lumen of the rosette (Fig 5B). These bulblike extensions contained numerous microtubuli and centrioles, which often differentiated into basal bodies (Fig 5B), sometimes with cilia showing the typical 9 + 2 pattern of microtubuli (Fig 5B, inset). The similarity of these structures to dendritic receptors of olfactory sensory cells (olfactory vesicles) is striking, though in tumors we see them in neoplastic distortion. Because of the irregular growth pattern of tumors, these receptor structures of olfactory sensory cells develop not only on the surface of rosettes, but likewise in the compact tumor area itself. Figure 5C shows densely packed cell processes with multiple basal bodies and a solitary cilium in the perinuclear cytoplasm of an adjacent cell.

In other areas of these rosettes, there were numerous distended axons containing neurotubuli and many electron-dense structures (Fig 6A) that most likely represent degenerated mitochondria (Fig 6B). Inside these axons there were also smaller membrane-bound structures resembling synaptic vesicles (Fig 6C). Besides these structures with the differentiation of olfactory sensory cells, other cells inside these rosettes resembled supporting cells of olfactory epithelium (Fig 6D). In addition to microvilli and tight junctions, they contained — as do normal supporting cells — desmosomes and tonofilaments.

In our previous report[25] we showed that in rare cases this tumor differentiates into squamous cell carcinoma. The ultrastructural aspect of this differentiation is shown in Fig 6E. In the upper part there are undifferentiated cells with big nuclei and scanty cytoplasm. In the lower right of Fig 6E, cells of the same type show a dramatic formation of tonofilaments.

DISCUSSION

As shown in our previous report,[25] the induced tumors were composed of undifferentiated small round cells lacking any special diagnostic characteristic except their marked tendency to create different rosette formations. In particular, neuroblastic Homer Wright rosettes[28] and "ependymal" Flexner rosettes[29] were encountered, which have likewise been described in human esthesioneuroepitheliomas,[23,24,30] as well as in experimentally induced tumors.[15-17,31] However, rosettes themselves

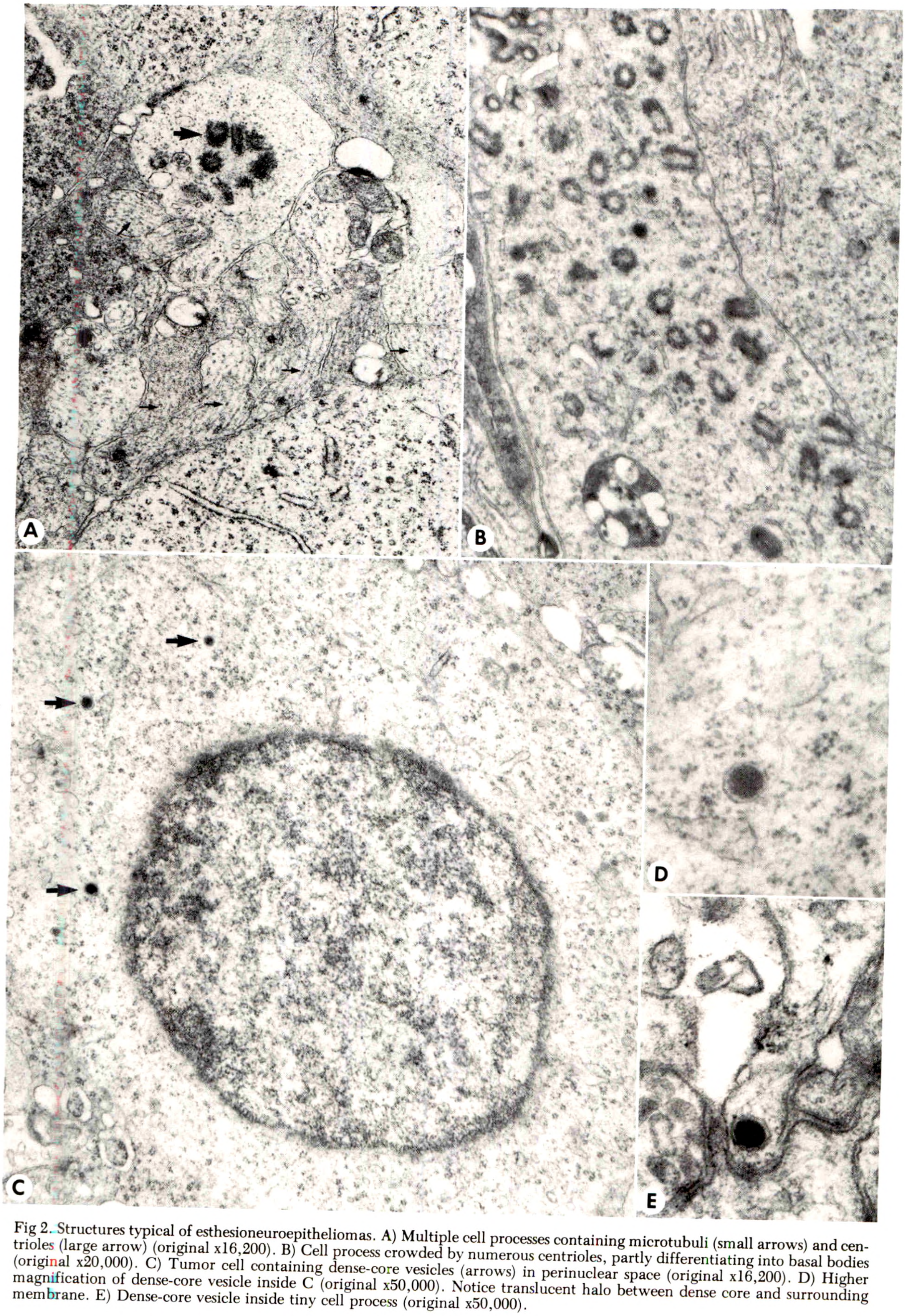

Fig 2. Structures typical of esthesioneuroepitheliomas. A) Multiple cell processes containing microtubuli (small arrows) and centrioles (large arrow) (original x16,200). B) Cell process crowded by numerous centrioles, partly differentiating into basal bodies (original x20,000). C) Tumor cell containing dense-core vesicles (arrows) in perinuclear space (original x16,200). D) Higher magnification of dense-core vesicle inside C (original x50,000). Notice translucent halo between dense core and surrounding membrane. E) Dense-core vesicle inside tiny cell process (original x50,000).

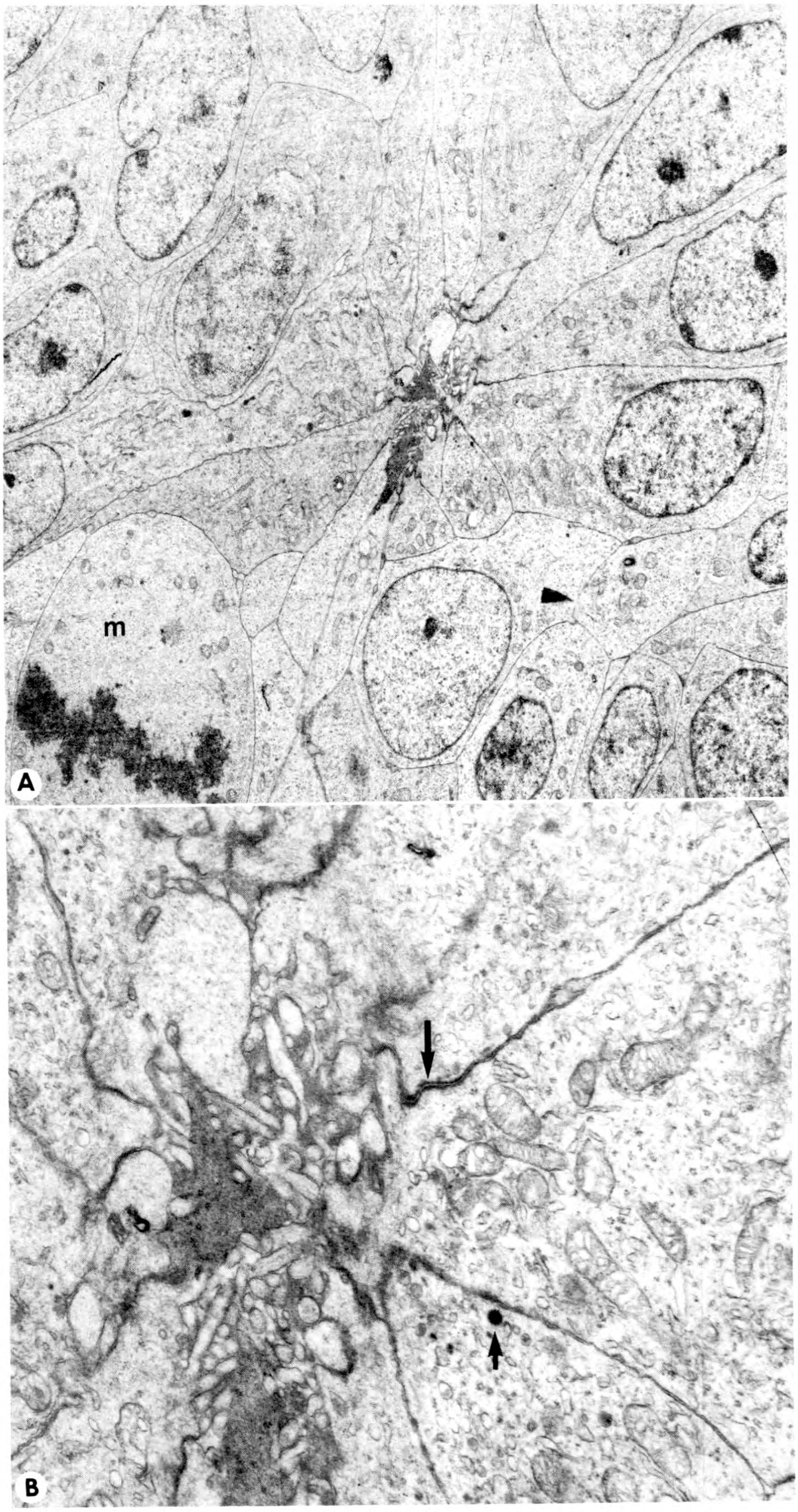

Fig 3. Homer Wright rosette. A) Overview (original x16,000). Elongated cytoplasmic cell apices converge toward central lumen, which is filled with amorphous material. Note undifferentiated neuroblastic nature of cells. m — cell undergoing mitosis. B) Central lumen (original x34,000). Cell apices are linked together by tight junctions (long arrow). Lumen is filled with amorphous material and contains some microvilli. Note dense-core vesicles (short arrow).

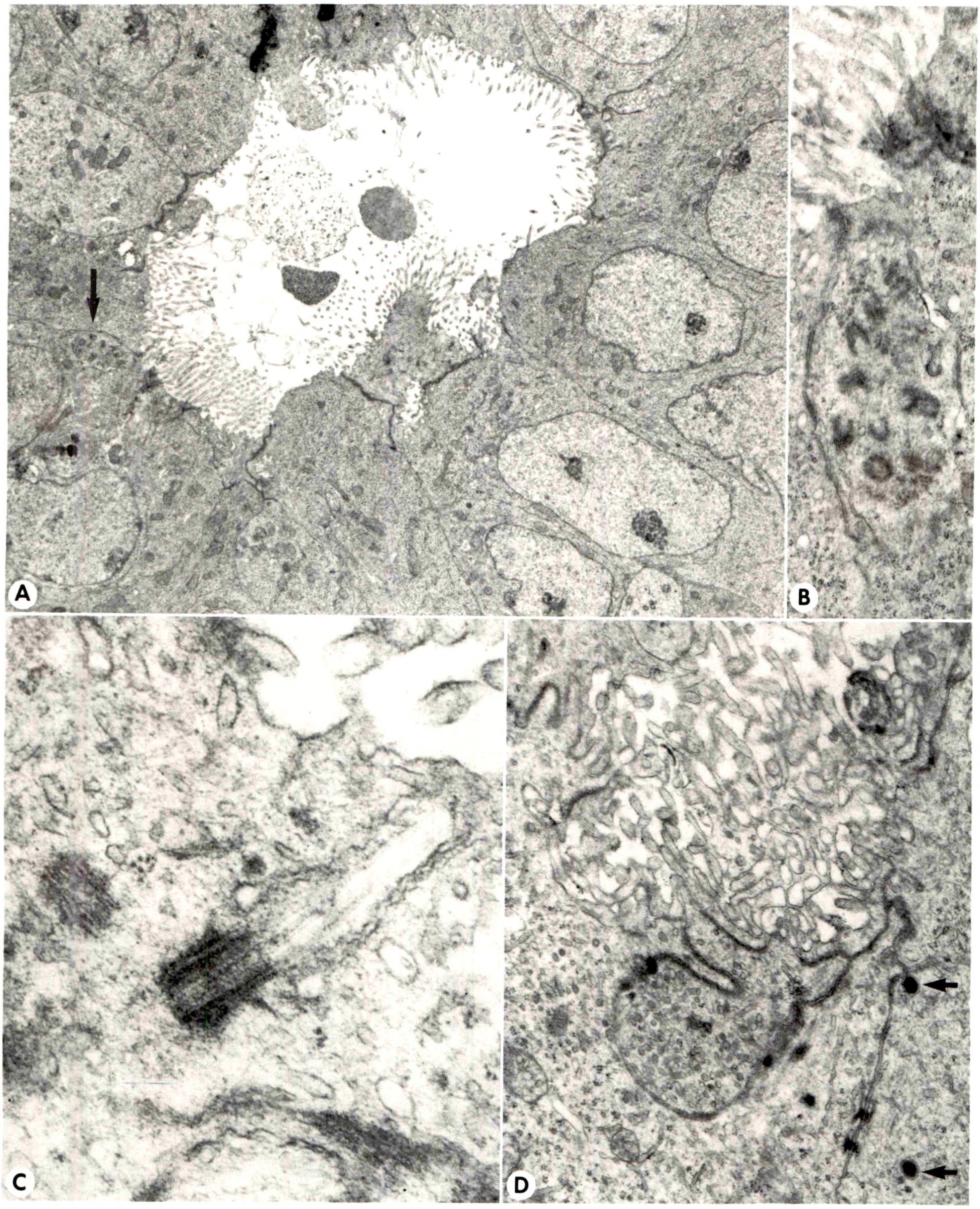

Fig 4. Flexner rosette. A) Cuboidal cells surround wide central lumen (original x5,800). Cell surfaces are covered with microvilli. At left side, cell process containing centrioles is approaching lumen (arrow). B) Indicated cell process of A at higher magnification (original x40,000). Contains typical centrioles sometimes differentiating into basal bodies. C) Basal body close to lumen with outgrowing cilia (original x60,000). D) Cells at luminal border are linked together by junctional complexes (original x24,000). Note neurosecretory granules, ie, dense-core vesicles (arrows).

are not pathognomonic for this kind of tumor, since they are found also in cerebral neuroblastomas, medulloblastomas, retinoblastomas, ependymomas, cerebellar neuroblastomas, and neuroblastomas of the autonomic nervous system.[32] Ultrastructural investigations of the tumor cells and their rosette ar-

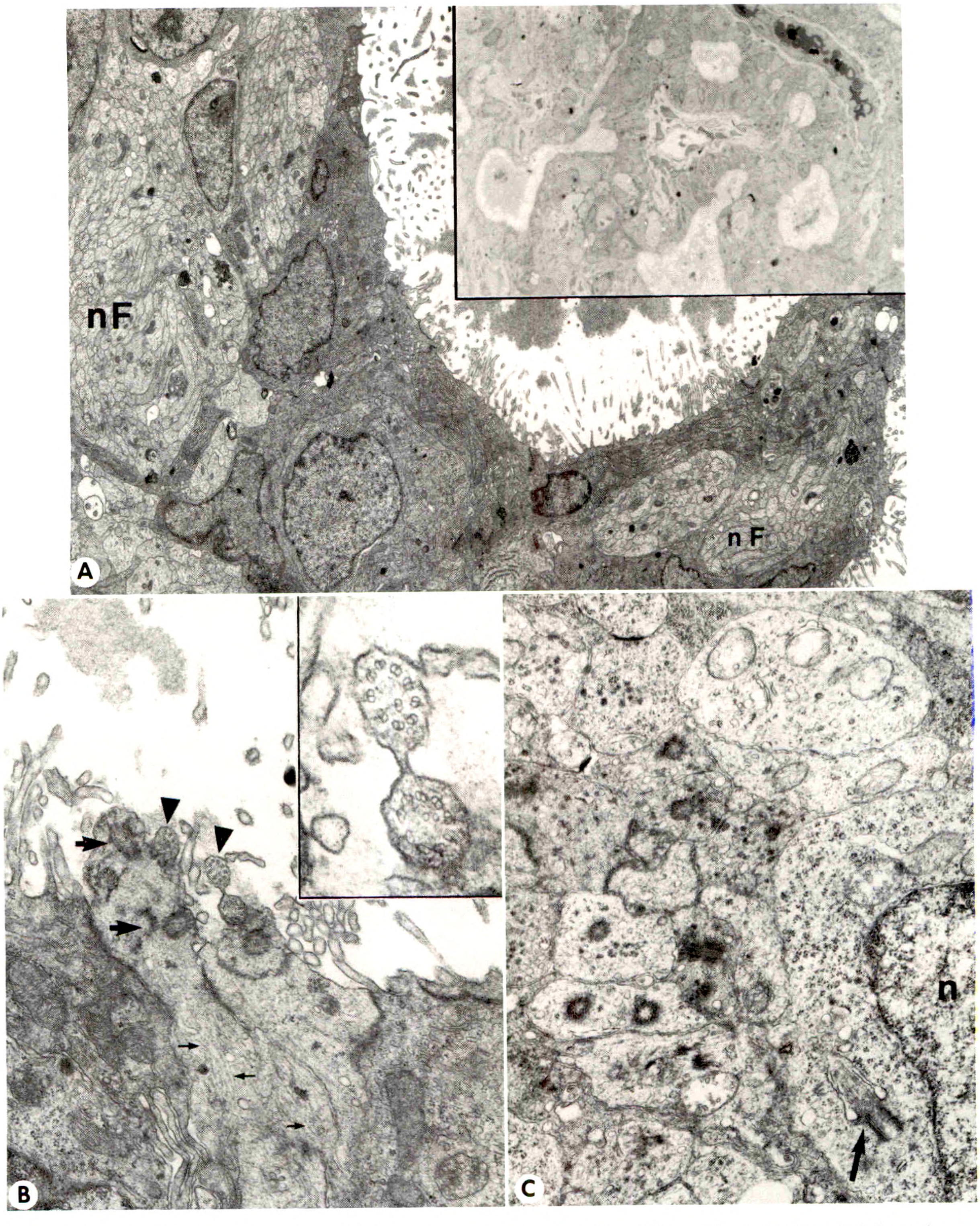

Fig 5. Distended Flexner rosette imitating glandular structures. A) Bundles of nerve fibers (nF) running irregularly beneath surface (original x4,800). Inset) Light microscopic appearance of distended rosettes (semithin section, toluidine blue, original x240). B) Cell processes protruding into lumen of Flexner rosettes showing characteristic features of olfactory vesicles (original x16,000). Basal bodies (large arrows), microtubuli (small arrows), and cilia (arrowheads) are seen. Inset) Abnormal cilia linked together and showing typical 9 + 2 arrangement of microtubuli (original x54,000). C) Cell processes under surface of rosette lumen containing basal bodies identical to those shown in B (original x16,800). Solitary cilium in perinuclear space (arrow). n — nucleus.

rangements are therefore necessary for a thorough classification of this tumor and for consideration of its histogenesis.

The ultrastructural appearance of the small round cell within the compact tumor area is in fact highly similar to both those of peripheral sympa-

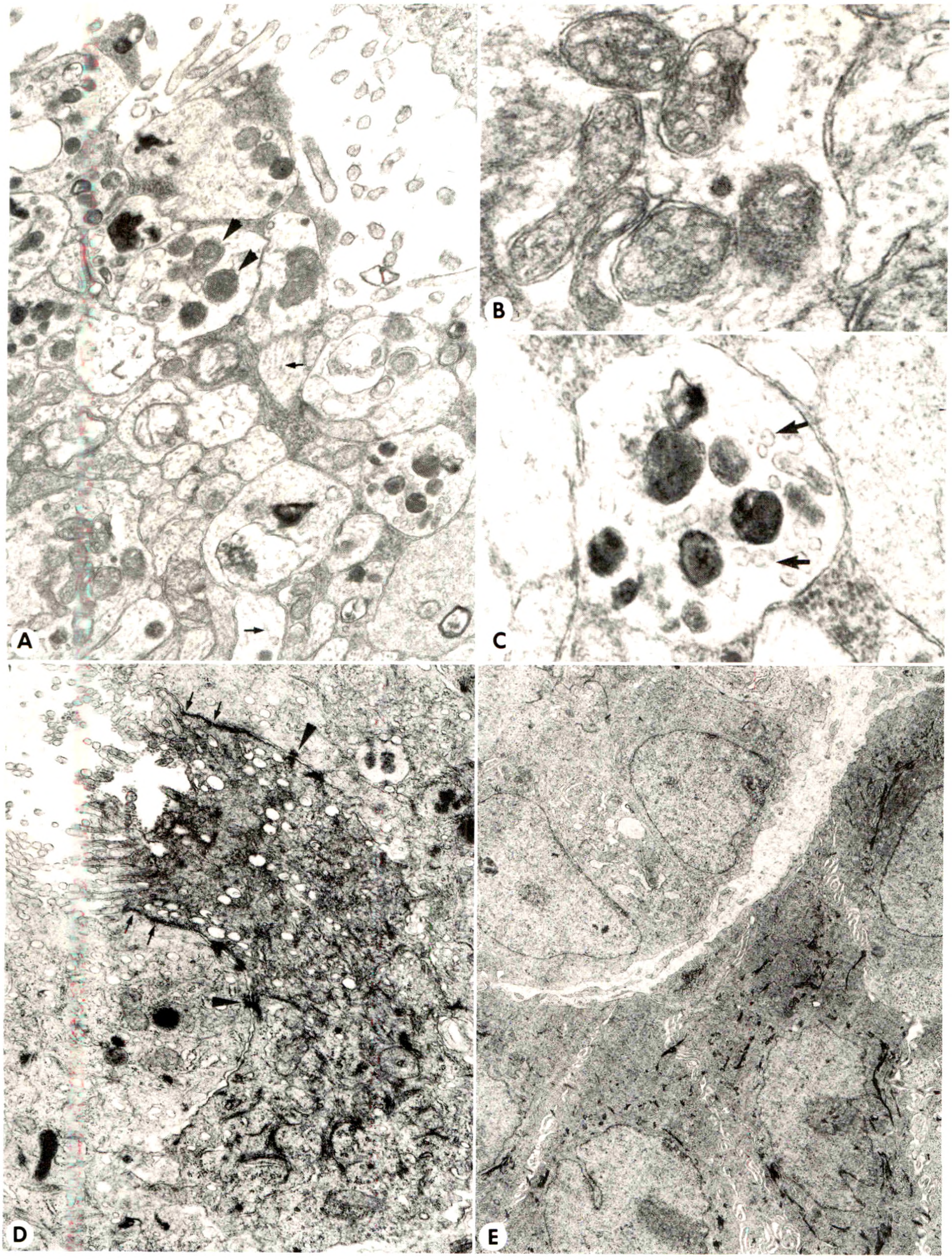

Fig 6. Neuronal and epithelial differentiation of tumor cells. A) Axonal processes containing microtubuli (arrows) and degenerated mitochondria (arrowheads) (original x16,000). B) Degenerated mitochondria inside axonal processes (original x54,000). C) Synaptic vesicles (arrows) inside axonal processes (original x54,000). D) Cells of Flexner rosette showing epithelial differentiation: tight junction (arrows), desmosomes (arrowheads), and massive development of tonofilaments (original x16,000). E) Maturation of undifferentiated neuroblastic tumor cells (top) into epithelial cells of squamous cell carcinoma (bottom) with massive expression of tonofilaments (original x16,000). Note similarity of nuclei in both cell types.

thetic neuroblastomas[33] and those of neuroblastomas of the CNS.[24] All of these tumors are built of small undifferentiated round cell elements with a high nucleus/cytoplasm ratio. Even the rarely encountered specializations of cell membranes of closely packed cells are identical to those of intracerebral neuroblastomas as described by Hirano et al.[34] Dense-core vesicles are not pathognomonic for esthesioneuroepitheliomas, as originally believed by McGavran,[22] but a common finding in all the neuroendocrine tumors designated as APUD tumors by Pearse.[35] Though dense-core vesicles are only exceptionally found in normal olfactory mucosa,[36,37] their regular presence in esthesioneuroepitheliomas allows the latter to be classified as neuroendocrine tumors. This view has been substantiated by elevated levels of peptide hormones in tumor tissue and plasma, as described in our previous paper.[25] Thus the opinion of Fujita[38] and Kurosumi,[39] ranking olfactory sensory cells as paraneurons between neurons and polypeptide-producing cells, is justified.

The abundance of rosettes in these chemically induced tumors provided the opportunity for a thorough investigation of these cellular formations. Homer Wright rosettes are built of typically undifferentiated tumor cells whose apices are strongly elongated and linked together around the tiny central lumen. With the exception of the eccentrically located nucleus, these cells showed all characteristics of undifferentiated primitive neuroblasts, so that the term "neuroblastic rosette" is justified on the ultrastructural level.[32] The central lumen was filled with amorphous material and microvilli originating from the cell tips.

All aspects of these rat tumor rosettes are identical with those in human tumors, as for instance those in cerebral neuroblastomas[26] and in sympathetic neuroblastomas.[40] More characteristic of this tumor, however, were the ultrastructural findings inside the Flexner rosettes. The cells of these rosettes are linked by tight junctions, the equivalent of the internal limiting membrane at the light microscopic level.[25] Highly typical of this type of tumor were the bulblike cell extensions protruding into the lumen of the rosette, clearly representing olfactory vesicles in neoplastic distortion. As do normal olfactory vesicles,[41] these processes contain basal bodies, olfactory cilia, and microtubuli arranged in parallel fashion. However, even inside the compact tumor far from any rosettal lumen, the tumor cells expressed such characteristic features as multiple centrioles, microtubuli, and even cilia. Whereas these structures represent the dendritic receptor end of olfactory sensory cells, in other parts of the tumor there were tumorous axon sproutings protruding into the rosette. Besides synaptic vesicles, these axons contained conglomerates of dying mitochondria accumulating in blind sac endings of regenerating axons, which have also been identified in regenerating axons of other tissues.[42-44] Though normally in regeneration experiments of the olfactory epithelium there is no isolated axonal sprouting,[41] the similar findings might be explained by the fact that the outgrowing axons of neoplastic olfactory sensory cells do not come into contact with the olfactory bulb. Because of the great similarity of cellular structures inside cells of the Flexner rosettes in CNS tumors, many authors have said that the tumors within these rosettes are imitating the cellular architecture of the normal mucosal lining of the neural tube.[32,45]

This interpretation is supported strikingly by our ultrastructural findings, with the following difference: in this tumor Flexner rosettes imitate the mucosal lining of the olfactory placode, since the olfactory epithelium is of placodal origin. The Flexner rosettes with cells showing rudimentary olfactory vesicles containing basal bodies, cilia, and microtubuli, the interconnection of adjacent cells by tight junctions, and other cell types containing microvilli are indeed a neoplastically altered but unmistakable image of the apical (surface) portion of the normal olfactory epithelium. The equally distinctive neoplastic axon convolutions correspondingly represent the basal portions of the olfactory epithelium.

Since it is generally accepted that tumors are derived from a single, primarily deteriorated cell,[46] the question arises as to which cell type inside the olfactory epithelium is the stem cell of this tumor. Since the ultrastructural characteristics of tumor cells are highly similar to those of the light basal cells inside the olfactory epithelium,[47] it is very likely that these cells, which have a regenerative capacity throughout a lifetime,[41] are the stem cells of this type of tumor. Reznik-Schüller[48] recently used another carcinogen to induce similar tumors in the olfactory epithelium of rats, which she believed to be derived from olfactory cells.

Finally, according to the monoclonal stem line concept of tumor development, the demonstration of squamous cell carcinoma in this kind of tumor implies that the basal cells of the olfactory epithelium are pluripotent cells that — at least in neoplastic deterioration — may develop either into sensory cells or into epithelial (supporting) cells, since both cell types were seen in the tumors. The first pathway would initiate the described undifferentiated "neurogenous" esthesioneuroepithelioma; the second would lead to a squamous cell carcinoma. Support for this view of the light basal cell as a pluripotent undifferentiated stem cell has been given in our previous report on normal olfactory epithelium.[47] In that report we described normal dark basal cells containing both tonofilaments and multiple centrioles with differentiation into basal bodies. A similar observation of an equally qualitative change at the cellular level of chemically induced tumors within the bronchial mucosa has been reported by Reznik-Schüller.[12] Though some of the presented findings were observed in transplants of the primary rat tumor to nude mice, the surprising results, for instance epithelial differentiation, must not be misinterpreted as being caused by this nude mouse passage. First, our ultrastructural as well as immunohistologic findings in primary rat tumor and nude mouse transplant were identical in all cases. Second, it has been well documented that nude mouse tumors do not change their character during serial passage,[49,50] but keep their typical features even down to the chromosomal level.[51]

Analogous to the extensive light microscopic and immunohistologic likeness of chemically induced tumors of the olfactory epithelium to human esthesioneuroblastomas,[25,52] there is also great agreement between both the human and the experimentally induced tumors on the ultrastructural level. Thus, identical neurosecretory granules were observed in human esthesioneuroblastoma.[21-24] "Neuritic" processes have been described by Mackay et al,[24] Chaudhry et al,[21] and Vollrath et al.[52] Neurotubuli were described by several authors.[21,23,24] A striking proof of the similarity between human esthesioneuroepithelioma and an experimental tumor was the demonstration of olfactory vesicles with centrioles inside a Flexner rosette of two human esthesioneuroblastomas by Mackay et al[24] and Silva et al.[53] Because of this overwhelming similarity, which likewise is paralleled on an immunohistologic basis,[25] it seems justified to call these chemically induced rat tumors esthesioneuroepitheliomas. This finding confirms the early conclusions of Thomas[15,16] and Herrold and Dunham,[8] which were drawn on the basis of light microscopy alone. One explanation for this outstanding conformity between a human and a rat tumor may be the fact that the structure of the olfactory epithelium shows only minor changes throughout evolution, so that it appears to be an ancestral heritage of humans.[44,54] In striking support of this view is the existence of spontaneously occurring esthesioneuroepitheliomas in a cat,[55] and especially in a domestic carp,[56] with identical ultrastructural features.

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PHARYNGEAL FISTULAS IN POSTOPERATIVE HYPOTHYROID PATIENTS

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Hypothyroidism is a well-recognized complication of combined treatment for head and neck cancer. We discuss four patients who developed pharyngeal fistulas after total laryngectomy and preoperative (one patient) or postoperative (three patients) radiation therapy. The fistulas were refractory to conservative and surgical repair. Once the existence of hypothyroidism was established, immediate substitution therapy resulted in rapid healing of the fistulas and marked improvement of the patients' general condition. Few clinical reports exist on postoperative hypothyroidism and wound healing complications. Experimental work shows delay and impairment of wound healing in hypothyroid animals as opposed to hyperthyroid ones, in which wound healing was accelerated. Suspicion is warranted in patients after treatment for head and neck cancer when they exhibit even minimal symptoms of hypothyroidism or develop postoperative complications refractory to conservative treatment.

KEY WORDS — hypothyroidism, pharyngeal fistula, wound healing.

INTRODUCTION

Hypothyroidism is a well-recognized complication of therapy for carcinoma of the head and neck region. Zohar et al[1] reviewed 144 patients after carcinoma therapy and found 3.5% to be chemically and clinically hypothyroid. Hypothyroidism was found mainly in patients who underwent total laryngectomy and hemithyroidectomy combined with postoperative irradiation. Vrabec and Heffron[2] studied the effect of radiation therapy and surgery on thyroid function. Serum thyroid-stimulating hormone (TSH) levels were obtained as a screening test. In this study 29.1% of patients were found to be hypothyroid, the highest incidence (66%) of this condition occurring in the group treated by combined irradiation and surgery. Other authors found thyroid hypofunction in 20%[3] to 24%[4] of their patients after combined treatment for head and neck cancer.

We report on four patients who had undergone combined treatment for squamous cell carcinoma of the larynx and developed treatment-resistant pharyngeal fistulas. Three of the patients were operated on in our department and another was referred for treatment from another hospital. All patients were men (mean age, 67 years).

CASE REPORTS

Case 1. The patient, 72 years of age, had a stage III carcinoma of the larynx. A total laryngectomy and a left radical neck dissection were performed. External postoperative radiotherapy (5,000 R) was administered. For 5 months posttreatment he displayed fatigue and depression. We observed a puffy edematous face with severe flap edema and a pharyngeal fistula. Despite conservative and repeated surgical procedures the fistula failed to heal. Laboratory tests revealed hypothyroidism (thyroxine [T4], 2 μg/dL; thyroid-stimulating hormone [TSH], 29 mU/mL; normal values — T4, 4.5 to 12.5 μg/dL; TSH, 1.5 to 6 mU/mL). Substitution hormone therapy was started (levothyroxine [L-thyroxine], 0.25 mg/d). The patient's facial edema resolved and his general condition improved. The fistula was closed successfully by a pectoralis major myocutaneous island flap.

Case 2. The patient, 59 years of age, had a stage III carcinoma of the larynx. He underwent a total laryngectomy with only 2,700 R of postoperative irradiation because he refused to continue treatment. He developed a pharyngeal fistula 2 months after surgery. Conservative treatment yielded no significant improvement in his condition. Facial edema and lethargy slowly developed. Three months after surgery, laboratory tests revealed hypothyroidism (T4, 1.8 μg/dL; TSH, 20 mU/mL). Rapid improvement was noted after initiation of L-thyroxine therapy, and the fistula completely healed with some minor local curettage.

Case 3. The patient, 69 years of age, had a stage III carcinoma of the larynx. He had total laryngectomy and right neck dissection, and a small pharyngeal fistula developed during the first 3 weeks after the operation. Conservative attempts at repair were not successful because of the patient's poor general condition. The neck flaps and the peristomal region became puffy and edematous. Laboratory tests showed free thyroxine levels (FT4) to be 0.5 mg/dL (normal value, 0.8 to 1.6 mg/dL), and the TSH level was 27 mU/mL. Substitution therapy was started with rapid regression of the local edema and spontaneous closure of the fistula. The patient received 6,000 R of radiation treatment with no significant side effects.

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Case 4. The patient, 68 years of age, had a stage II carcinoma of the larynx. The patient received a treatment of 6,000 R and underwent total laryngectomy and near-total thyroidectomy 6 months later because radiation therapy failed. A pharyngeal fistula resistant to conservative treatment was noted 12 days after surgery. Operative attempts that included a trial with a deltopectoral flap failed to close the fistula. At this stage the patient was depressed and threatened to commit suicide. His pulse of 58 beats/min was attributed to beta-blocker treatment. Treatment with antidepressants resulted in some improvement of his mood. Locally no change was seen. FT4 levels of 0.4 mg/dL were found. L-thyroxine treatment (0.15 mg/d) resulted in a remarkable improvement. His feeling of well-being, appetite, and general condition greatly improved and the fistula closed rapidly.

DISCUSSION

Factors influencing wound healing after major head and neck operations include preoperative irradiation, operative technique and duration, the patient's nutritional state, and local infection. Thyroid dysfunction may impair wound healing as well. Multiple mechanisms are involved, such as protein and nucleic acid synthesis, mitochondrial oxidative phosphorylation, and the Krebs cycle enzyme system.[1,5] Few reports on hypothyroidism and wound healing can be found in the recent literature.

Alexander et al,[4] reviewing a series of patients after surgical therapy and irradiation to the head and neck and discussing wound healing in such conditions, reported three patients with treatment-resistant fistulas that closed after treatment with levothyroxine sodium. They conclude that wound healing, delayed in the hypothyroid state, can be accelerated with appropriate replacement therapy.

A 2-year delay in healing of a mandibular fracture was reported.[6] Once thyroid hormone supplementation was introduced, the fracture healed. Ladenson et al[7] studied the incidence of perioperative and postoperative surgical complications in preoperative hypothyroid patients. Of 40 patients, 17 underwent cardiac surgery while 23 had other operative procedures done. No difference was found in the frequencies of impaired wound healing or postoperative infection between hypothyroid and control patients.

The influence of hypothyroidism and hyperthyroidism on wound healing of experimental myocardial infarction in rats was studied by Kranz et al.[8] They found that hyperthyroidism accelerates wound healing, increases the number of DNA-synthesizing connective tissue cells, and shortens cell cycles. Release of tropocollagen by the fibroblasts takes place at a "marked increased rate." In hypothyroidism wound healing is delayed and release of tropocollagen is slowed down. Lennox and Johnston[9] studied healing rates and tensile strength of wounds in rats with varying levels of thyroid activity. Wound healing was accelerated by a mean of 2.5 days in the hyperthyroid group and delayed by 2.0 days in the hypothyroid group as compared to a control group. The tensile strength of linear wounds was significantly greater at 10 days in the hyperthyroid group. Basal nitrogen turnover increased and correlated with accelerated wound healing.

Mehregan and Zamick[10] demonstrated that triiodothyronine had a beneficial effect on the healing of deep thermal burns in rats. Herndon et al[11] showed that levels of thyroid hormone have a profound effect on the healing of wounds in thyroidectomized guinea pigs.

Our patients demonstrated marked rapid improvement in their general condition and wound healing after initiation of substitution therapy. Our experience supports reports concerning delay and impairment of wound healing in hypothyroid patients and in experimental models. Patients undergoing surgical treatment with or without irradiation for head and neck tumors should be evaluated preoperatively and postoperatively for thyroid function. Suspicion is warranted as symptoms and signs may be minimal or absent. Prompt diagnosis and treatment can be the key to an uninterrupted postoperative course.

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SURGICAL EXPERIENCE WITH JUVENILE NASOPHARYNGEAL ANGIOFIBROMA

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Our experience with 18 cases of juvenile nasopharyngeal angiofibroma (JNA) over a period of 9 years is discussed. All cases were managed surgically: 17 via a transpalatal approach and one case by a combined transpalatal, lateral rhinotomy, and transantral approach. In this series there were two recurrences following primary surgical procedures and these required a second procedure. There were no major complications and all 18 patients are alive. We believe that surgical excision should be the treatment of choice for JNA and that radiotherapy should be used adjunctively for cases wherein intracranial extension of the tumors prevents total excision.

KEY WORDS — juvenile nasopharyngeal angiofibroma.

INTRODUCTION

Juvenile nasopharyngeal angiofibroma (JNA) is a benign, highly vascular tumor that predominantly affects male adolescents. It accounts for less than 0.05% of head and neck tumors, although it is noted to be the most common benign tumor originating in the nasopharynx.[1,2] Although histologically benign, the tumor is locally invasive and occasionally penetrates the cranium.

This tumor has a tendency to grow and extend along the natural foramina and fissures associated with the site of origin in the nasopharynx.[2] It usually does not invade bone, but may erode it by pressure. Conley et al[2] stated that although a JNA can destroy the bone of the skull base, it usually does not invade the dura mater, leaving a useful cleavage plane for the surgeon.

Management of angiofibromas has been a challenge to the head and neck surgeon, and during the past century, treatment modalities have included surgical excision, electrocoagulation, interstitial or external radiation therapy, cryosurgery, and hormone administration. Only surgical and radiation treatments seem to be effective.

With the currently improved surgical approaches and adjuvant preoperative techniques of reducing surgical blood loss, most experts[1-9] prefer surgical resection as the primary modality of treatment for extracranial JNA, incorporating postoperative radiotherapy in those cases with unresectable intracranial extension.

PATIENTS AND METHODS

During the 9-year period April 1978 to March 1987, we diagnosed and managed 18 patients with JNA in the Durban teaching hospitals. All the patients were males between the ages of 11 and 23 years, with an average age of 16 years (see Table). The racial distribution of the patients was as follows: Indians, eight; Africans, six; whites, three; and Chinese, one.

The main symptom in 11 patients was recurrent bouts of spontaneous epistaxis. All the patients complained of some degree of nasal obstruction, and five patients had bilateral total nasal obstruction. Other symptoms included eustachian tube dysfunction, rhinolalia, conductive deafness, and headaches.

Diagnosis. All patients were subjected to a thorough otorhinolaryngologic examination. Sixteen patients revealed a fleshy mass on posterior rhinoscopy and seven had a fleshy polyp on anterior rhinoscopy. The findings on clinical examination suggested a high probability of JNA in all cases. We do not, as a rule, perform biopsies on such lesions, but instead subject the patients to the following radiographic investigations to confirm our clinical impressions: plain sinus views, submentovertical view, computed tomography, and carotid angiography.

While conventional radiography, such as plain sinus views and base of skull view, offers valuable information regarding the tumor, the use of CT scanning and selective internal and external carotid angiography is invaluable in assessing the exact extent of the lesion and confirming the diagnosis. In addition, the carotid angiograms show the major feeding vessels to the lesion and delineate the periphery of the tumors. On the plain films, anterior "bowing" of the posterior wall of the maxillary sinus is the most consistent and reliable finding associated with these tumors.[3]

In all our cases, the tumors received their major blood supply from either the internal maxillary or the ascending pharyngeal branches of the external carotid system. In two cases, the tumors received a significant blood supply, in addition, from the con-

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PATIENT DATA

Pt	*Age (yr)*	*Surgical Approach*	*Estimated Blood Loss (mL)*	*Blood Transfusion (units)*	*Hospital Stay (d)*
1	16	TP	600	2	6
2	17	TP	200	0	6
3	11	TP	700	2	7
4	22	TP	200	0	6
5	12	TP	800	1	9
6	16	TP	300	0	7
7	23	TP	300	2	6
8	12	TP	800	2	8
9	15	TP	400	1	5
10	16	TP	650	2	6
11	19	TP	550	2 + 1	11
12	17	TP	400	2	7
13	15	TP	700	2	7
14	18	TP	200	0	5
15	13	TP	900	2	6
16	16	TP	200	0	5
17	18	TP	400	1	5
18	12	TP, TA, LR	2,500	6	12

Patients 1, 3, 7, 9, 10, 14, 16, and 17 were Indian; patients 2, 4, 5, 6, 8, and 15 were African; patients 11, 12, and 18 were white; and patient 13 was Chinese.

TP — transpalatal, TA — transantral, LR — lateral rhinotomy.

tralateral internal maxillary arteries — hence the importance of doing bilateral angiography. There were no complications following angiography in this series.

Management. We believe that surgical resection is the treatment of choice for all extracranial JNAs and that radiotherapy should be used as an adjunct for those cases with intracranial extensions that prevent complete excision of the tumors. Since there were no patients with intracranial extensions in this series, radiotherapy was not used in any of our cases.

Currently, several surgical approaches have been reported to gain access to the neoplasms, namely the transpalatal approach, Caldwell-Luc antrostomy, sublabial approach around the maxillary tuberosity, lateral rhinotomy, and transethmoid, transmandibular-pterygoid, and suprahyoid transpharyngeal approaches.[3]

In this series all tumors were removed by the transpalatal approach, except that in case 18 (see Table), which required a combination of the transpalatal, transantral, and lateral rhinotomy approaches to achieve complete removal of the neoplasm. In this case, the tumor had spread to the pterygopalatine fossa, the infratemporal fossa, the maxillary antrum, and the ethmoid sinuses on the left, and the ethmoid and sphenoid sinuses on the right (Figs 1 and 2). The transpalatal approach gives excellent exposure to the nasopharynx as well as to extensions into the sphenoid sinus and posterior nasal fossae. We used the U-shaped palatal incision based posteriorly and, when necessary, removed bone from the posterior part of the hard palate to gain adequate exposure to the anterior extent of the tumor.

Adequate exposure is essential, and piecemeal removal of tumor should be avoided as far as possible in order to minimize bleeding. Once the tumor has been completely exposed and mobilized, further mobilization is carried out in a subperiosteal plane along the posterior pharyngeal wall until the entire tumor is pedicled on its superior attachment. After total removal, bleeding usually is controlled easily with temporary packing. We routinely pack the wound with a firm Whitehead's varnish pack prior to closure of the palatal incision. The pack is removed after 48 hours.

Estimated blood losses varied from 200 to 2,500 mL, with an average loss of 600 mL. Preoperative estrogen therapy in the form of diethylstilbestrol, 5 mg thrice daily, for 6 weeks was used routinely in

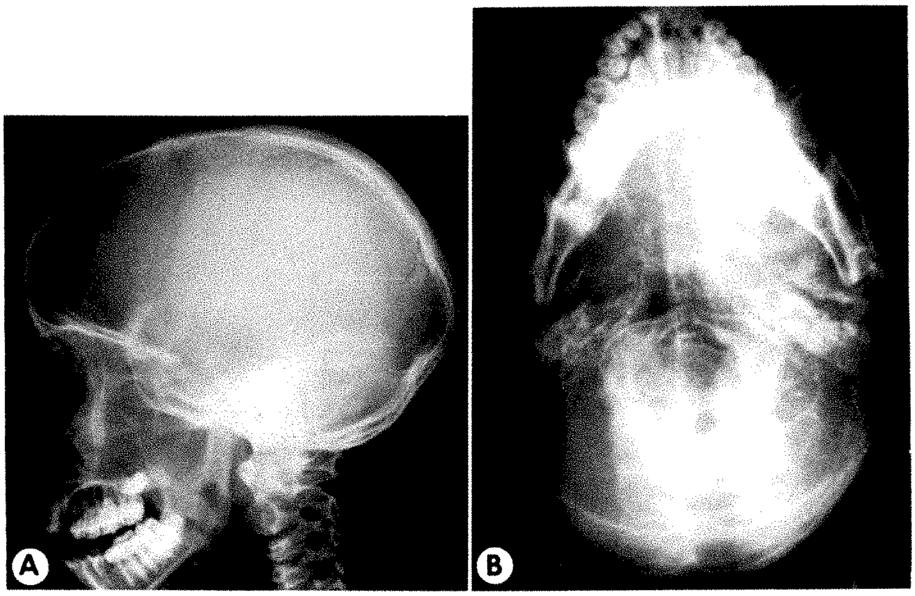

Fig 1. (Patient 18) A) Lateral plain film showing soft tissue mass in nasopharynx. B) Plain film submentovertical view showing large soft tissue mass in nasopharynx, extending to paranasal sinuses.

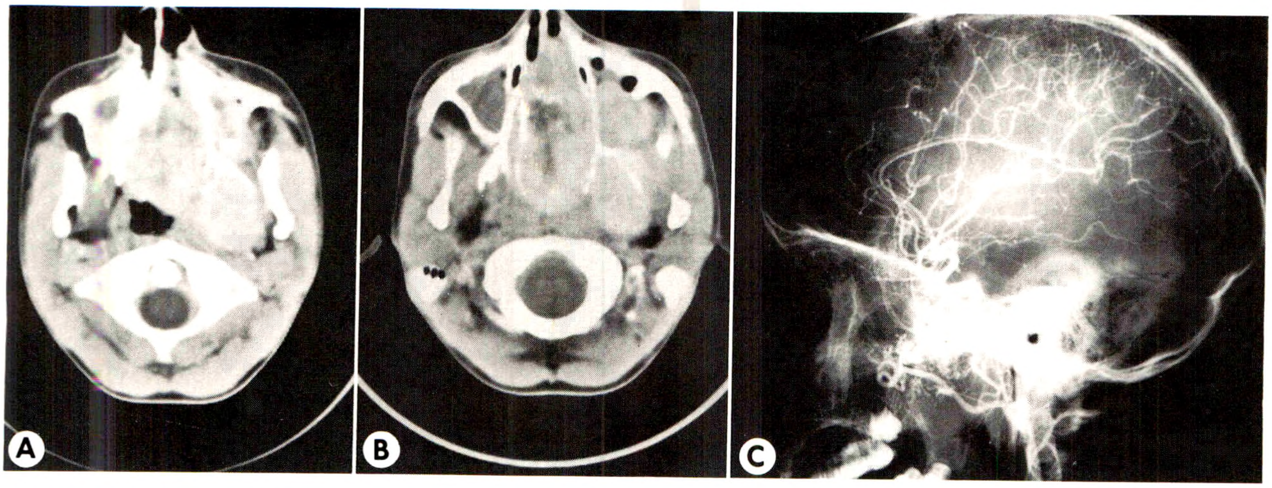

Fig 2. (Patient 18) A,B) Computed tomographs, axial view. A) Tumor in left nasal cavity, maxillary sinus, nasopharynx, pterygomaxillary space, and ethmoid sinuses. Note splaying of left pterygoid plates and anterior "bowing" of posterolateral wall of left maxillary antrum. B) Extension of mass into left infratemporal fossa and right ethmoid sinuses. C) Left carotid angiogram shows dilatation and anterior displacement of internal maxillary artery with feeder vessels to tumor.

all our cases. This, in addition to the hypotensive anesthetic used at surgery, appears to have helped in reducing the intraoperative blood loss. Neither preoperative embolization nor ligation of the major feeding vessels was attempted in any of our cases. The average perioperative blood transfusion was 1.8 units, and the average hospital stay, 7 days (see Table).

There were no major complications postoperatively. One patient had a secondary hemorrhage on the fifth postoperative day; he received a transfusion with another unit of blood and had to be repacked in the operating room under general anesthesia. One patient developed a small palatal fistula that closed spontaneously after 8 weeks. Our follow-up period extends from 3 months to 9 years. Sixteen patients have been followed up for over 2 years. There were two recurrences in this series. Patient 1 had a recurrent tumor after 5 years, and patient 10 had a recurrence after 6 months. In both these cases the recurrent tumor was removed surgically and bleeding was minimal. Both patients have been followed up for 3 years since the second procedure and neither has had any further recurrence. All our patients are alive and well.

DISCUSSION

The origin and pathogenesis of JNA remains obscure. Theories relate to its site of origin and histologic appearance. Ringertz[10] suggested that the tumor arose from the periosteum of the base of the skull. Osborn and Sokolovski[11] suggested that the tumor either was a hamartoma or arose from a remnant of fetal erectile tissue. Harma[12] thought that it was a hyperplastic tissue reaction caused by some hormonal factor. Walike and Mackay,[13] using the electron microscope, found that diethylstilbestrol changes the lining of vascular spaces and stimulates fibrosis and regression of the angiofibroma. Girgis and Fahmy[14] suggested that the tumor arises from nonchromaffin paraganglionic cells present at the terminal end of the maxillary artery. Hubbard[15] considers the JNA to be a distinctive type of hemangioma. Sessions and Humphreys[16] believe that this neoplasm arises from a broad base on the posterolateral wall of the nasal cavity in the area of the sphenopalatine foramen.

Histologically, the tumor is composed of spindle or stellate fibrocytes in some amount of connective tissue stroma. It contains thin-walled vascular channels, which differ both in size and distribution. The microscopic appearance may vary from one of erectilelike tissue or a cavernous hemangioma to that of a myxomatous fibromatosis.[17]

Sarcomatous changes have been described in a JNA, but are extremely rare.[9,18] Witt et al[9] and Batsakis et al[18] have each described a case of fibrosarcoma arising in a JNA following radiation therapy.

While JNA occurs predominantly in males, there have been reports of its occurrence in females.[11,17,19] Some authorities believe that it occurs only in the male and that if a female is involved, chromosomal studies and a reassessment of the histologic structure are necessary.[5]

There is general agreement by most authors that surgical resection is the treatment of choice for extracranial JNA, although some advocate radiotherapy as the primary treatment modality for these tumors.[18,20] Many clinicians reject radiotherapy as a treatment modality because of the remote chance of inducing an iatrogenic tumor or damaging normal structures and growth function. Briant et al[19] reported a control rate of 80% in their series of 45 patients treated with moderate dose radiotherapy. Others have used radiotherapy for surgically inaccessible intracranial extensions of tumor or for recurrences following a primary procedure.

With recent advances in surgical techniques, such as the combined intracranial-extracranial removal of tumors,[4,6-8] the use of CT and angiography to ascertain the exact extent of tumors, and the preoperative use of adjuvant measures such as embolization, estrogen therapy, and ligation of feeding vessels, coupled with hypotensive anesthesia, the successful removal of previously unresectable tumors has been made possible.

CONCLUSION

Our study supports the primary role of surgical management of JNA. In 18 patients with extracranial lesions who were followed up for 3 months to 9 years, we have a 100% cure rate (two of these patients required a second procedure). Except for one case in which the intraoperative blood loss was 2,500 mL, the average blood loss of 600 mL in our series compares favorably with those of other reported series.

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Ann Otol Rhinol Laryngol 98:1989

SECRETORY IGA AND BACTERIAL ADHERENCE TO NASAL MUCOSAL CELLS

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The ability of *Streptococcus pyogenes* to adhere to nasal mucosal cells was investigated by an in vitro assay system in order to clarify the actual role of secretory IgA in mucosal immunity in the nose. The number of bacteria adhering to isolated mucosal cells was significantly larger in patients with chronic sinusitis than in normal control subjects. The ability of bacteria to adhere, however, was significantly lower in the group of subjects having specific secretory IgA antibody activity to the M protein than in those having no secretory IgA activity. Findings demonstrated that mucosal immunity preventing or blocking the adherence of bacteria is impaired in patients with chronic sinusitis.

KEY WORDS — bacterial adherence, bacterial proteins, nasal mucosa, secretory IgA, *Streptococcus pyogenes*.

INTRODUCTION

Bacterial adherence is the initial step in the infection of the mucous membrane. The mechanism of bacterial adherence has been studied since 1971, when Gibbons and van Houte[1] reported an in vitro assay system to determine bacterial ability to attach to human epithelial cells. Subsequently, many investigators have studied the mechanism of bacterial adherence to mucosal cells of the mouth, intestine, and urinary tract. It now appears that bacterial adherence takes place between the adhesive molecules on bacterial surface and receptors of the host. However, few studies dealing with bacterial adherence to nasal mucosal cells have been reported.[2]

Secretory IgA is the most predominant immunoglobulin in external secretions, playing an important role in protecting the host against infection and other antigenic insult of the mucosal surface. Although it has been demonstrated that secretory IgA possesses antibody activities against various viruses,[3] the exact mechanism against bacterial infection still remains unclear. One postulated function is that secretory IgA is capable of blocking bacterial adherence to mucosal surfaces, preventing the colonization of bacteria. Williams and Gibbons[4] reported that secretory IgA isolated from parotid fluid specifically inhibited the adherence of *Streptococcus* strains to human buccal epithelial cells. However, the role of secretory IgA in bacterial adherence to nasal mucosa has not been investigated. Previously, we reported that secretory IgA antibody activity specific for the M protein, one of the cell wall antigens of *Streptococcus pyogenes*, was lower in patients with chronic sinusitis than in normal subjects.[5] The evidence suggested that the adherence of *S pyogenes* to nasal mucosal cells may be increased in patients with chronic sinusitis because the M protein has been considered to participate in its adherence.

In the present study, the adherence of *S pyogenes* to nasal mucosal cells isolated from patients with chronic sinusitis and from normal subjects was investigated in vitro. The results were compared to the amount of IgA concentration and the specific antibody activity of secretory IgA in nasal secretions in order to clarify the role of secretory IgA in bacterial adherence to nasal mucosal cells.

MATERIALS AND METHODS

Preparation of Mucosal Cell Suspension. Nasal mucosal cells were collected from 25 normal subjects and 29 patients with chronic sinusitis by swabbing the nasal mucosa of the anterior nares with a sterile cotton-tipped wooden swab. All of the samples were collected within an hour before the adherence test. The swabs were swirled in 0.067 *M* phosphate-buffered saline (PBS) pH 8.0 to dislodge the cells. The cells were washed with PBS to rid them of unattached bacteria by use of a membrane filter having a 12-μm pore size (Nuclepore Corp). The washed epithelial cell suspension was adjusted with PBS to contain from 2×10^4 to 4×10^4 cells/mL as determined by microscopic count. As preliminary tests, buccal mucosal cell suspensions were prepared from normal adult subjects in a similar manner. The age of patients with chronic sinusitis was between 13 and 70 years. Diagnosis of chronic paranasal sinus infection was based on findings of patient's history, rhinoscopic findings, and x-ray examination. Normal subjects, 14 to 61 years of age, were free from symptoms of nasal and other respiratory diseases and were judged to be normal by rhinoscopic examination.

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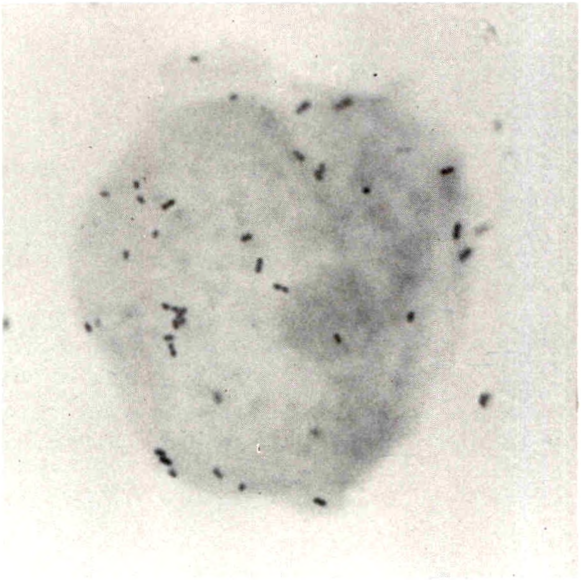

Fig 1. Demonstration of *S pyogenes* adherence to nasal mucosal cell (original x1,000). Mean number of bacteria attached per nasal mucosal cell was determined by direct microscopic enumeration of 20 mucosal cells in each subject.

Collection and Analysis of Nasal Secretion. Specimens of nasal secretion were obtained from each subject with the Juhn Tym-Tap (Xomed) at the same time the nasal mucosal cells were collected. To each sample, borate-buffered saline (BBS) containing 0.1% NaN_3 was added. It was mixed by a mixer and then centrifuged. The supernatant was stored at 4°C until used. Electroimmunodiffusion techniques as previously reported[5] were used to quantify secretory IgA and serum type IgA, and the antibody activity of secretory IgA in nasal secretion against the M protein was investigated by use of enzyme-linked immunosorbent assay (ELISA).

Preparation of Bacterial Suspension. Streptococcus pyogenes strains Sv type 3 (a virulent strain with M protein) and C203S (an avirulent strain without M protein) were donated by Research Laboratories of Chugai Pharmaceutical Co, Tokyo. After the virulence was increased by intraperitoneal mouse passage, strain Sv was maintained in 2 mL of Todd-Hewitt broth (THB; Difco) to which 10% fetal calf serum had been added. It was stored at -80°C. To prepare culture for the adherence test, one test tube of the sample was thawed for each test, inoculated into 2 mL of THB at 1:20 dilution, incubated overnight at 37°C, and transferred to 2 mL of THB at 1:10 dilution. After 5 hours of incubation at 37°C, the organisms were harvested by centrifugation and resuspended in PBS to a final density of 3 x 10^8 organisms/mL as determined by spectroscopy.

Adherence of S pyogenes *to Mucosal Cell in Vitro.* The adherence test was performed with a modification of the assay described by Gibbons and van Houte.[1] One milliliter each of mucosal cell preparation and bacterial suspension were mixed and incubated for 90 minutes at 37°C in a shaking water bath. After incubation, the mucosal cells were washed free of unattached bacteria in a 12-μm filter, by use of 40 mL of PBS. Direct smears were prepared from each mucosal cell suspension and stained with Gram's stain. The mean number of bacteria attached per mucosal cell was determined by direct microscopic enumeration of 20 epithelial cells at x1,000 magnification (Fig 1). Control mucosal cell suspensions incubated with PBS instead of bacterial suspension always were included to provide data on the number of bacteria that already were attached at the time of their collection. The difference between the mean number of bacteria attached to the mucosal cells mixed with S

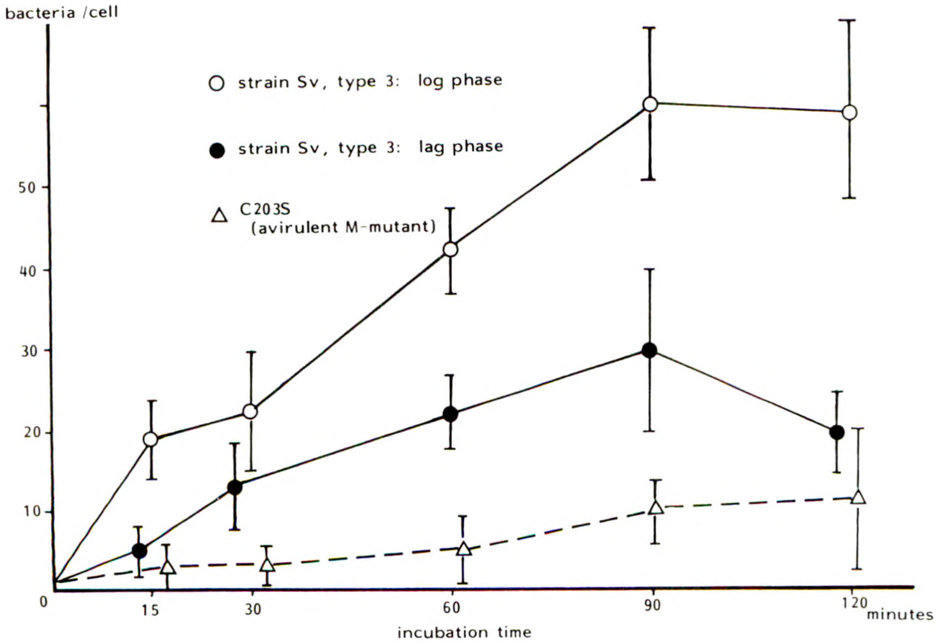

Fig 2. Adherence of strain Sv at midlog phase, strain Sv at lag phase (24-hour culture), and C203S to buccal mucosal cells. Adherence of strain Sv at midlog phase is greater than that of strain Sv at lag phase, or of C203S.

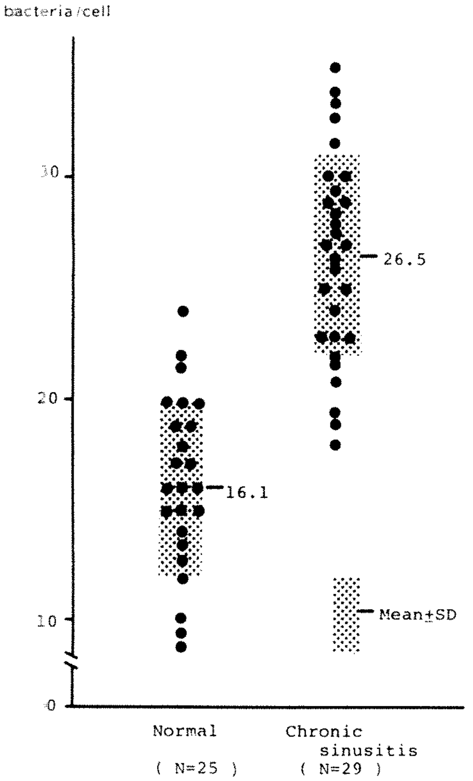

Fig 3. Adherence numbers of *S pyogenes* to nasal mucosal cells. Mean number of adherent bacteria is significantly greater in patients with chronic sinusitis than in normal subjects (p< .001).

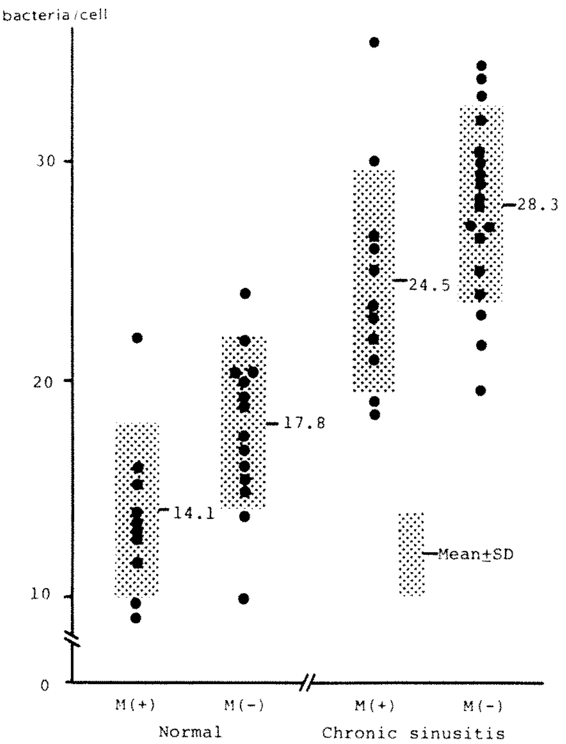

Fig 4. Secretory IgA antibody activity against M protein and bacterial adherence. Bacterial adherence in subjects having secretory IgA antibody activity is significantly lower than in those without antibody activity in both categories, normal subjects and patients with chronic sinusitis (p< .05, p< .05).

pyogenes and that of the control suspension was calculated.

RESULTS

Adherence of S pyogenes *to Buccal Mucosal Cells.* *Streptococcus pyogenes* strains Sv type 3 and C203S had log phase between 4 and 6 hours of incubation in 2 mL of THB at 37°C. Adherences of strain Sv at midlog phase, strain Sv at lag phase (24-hour culture), and C203S at midlog phase to buccal mucosal cells are shown in Fig 2. Adherence of strain Sv at midlog phase was greater than that of strain Sv at lag phase, or of C203S, and maximum adherence occurred at 90 minutes after incubation.

Adherence of S pyogenes *to Nasal Mucosal Cells.* The adherence numbers of *S pyogenes* strain Sv at midlog phase to nasal mucosal cells are shown in Fig 3. The mean number of adherent bacteria in normal subjects was 16.1±4.0 bacteria/cell and that in patients with chronic sinusitis was 26.5±4.6 bacteria/cell. This difference was statistically significant (p< .001).

The mean concentration of secretory IgA in nasal secretions was 200.6±154.5 mg/dL in normal subjects and 322.6±213.2 mg/dL in patients with chronic sinusitis. The mean concentration of serum type IgA in nasal secretions was 24.9±12.7 mg/dL in normal subjects and 30.0±13.1 mg/dL in patients with chronic sinusitis. Mean concentrations of both secretory IgA and serum type IgA in nasal secretions from patients with chronic sinusitis were greater than those from normal subjects, although these differences were not significant. However, these results are similar to those we reported previously.[5]

In each category, subjects were separated into two groups — high concentration and low concentration — by the mean values of secretory IgA and serum type IgA in nasal secretions. These were compared with the number of adherent bacteria. The numbers of adherent bacteria were similar to each other between the high concentration group and the low concentration group of both secretory IgA and serum type IgA in each category, and there was no significant difference.

Secretory IgA with specific antibody activity against the M protein was detected in nasal secretions in 11 normal subjects (44%) and in 11 patients with chronic sinusitis (38%). The percentage of samples possessing specific secretory IgA antibody activity was lower in patients with chronic sinusitis than in normal subjects, as previously reported.[5] The ELISA titers of secretory IgA and total IgA (secretory IgA + serum type IgA) in nasal secretions of patients with chronic sinusitis were slightly but not significantly lower than those of normal subjects in the present study. As shown in Fig 4, the mean number of adherent bacteria of subjects having secretory IgA antibody activity specific for the M protein was significantly lower than in those having no antibody activity. This finding was observed in both categories, normal subjects and patients with chronic sinusitis ($p<.05$, $p<.05$).

DISCUSSION

Streptococcus pyogenes, with an array of antigens, is one of the major causative agents of upper respiratory tract infections. M protein, one of the cell wall antigens associated with its virulence, has been considered to participate in the adherence of this organism to the mucosal surface. In the present study, the adherent ability in vitro of strain Sv at midlog phase, when the M protein is synthesized most actively,[6] was greater than that of strain Sv at lag phase, or than that of C203S (an avirulent strain lacking M protein) at midlog phase. These results support the finding of Ellen and Gibbons[7] that M protein mediates the adherence of *S pyogenes* to epithelial cells. On the other hand, Alkan et al[8] suggested that there are no differences in adherence between M protein–positive and M protein–negative strains of group A streptococci obtained from pharynx and skin. Beachey and Ofek[9] proposed that lipoteichoic acid (LTA), rather than M protein, participates in the adherence of *S pyogenes* to epithelial cells, because in the components of *S pyogenes* only LTA was able to block the adherence. However, Beachey[10] presented evidence that LTA and deacylated LTA form a complex network with M protein on the surface of *S pyogenes* that serves as an adhesin. From his investigation, it can be considered that M protein has some association with the adherence of *S pyogenes* to epithelial cells, although it remains unclear whether directly or indirectly.

It has been observed that epithelial cells from certain subjects have an increased susceptibility to bacterial adherence. Andersson et al[11] demonstrated the increased binding of pneumococcal strains to epithelial cells taken from patients with frequent attacks of otitis media compared to age-matched controls. Aly et al[2] reported that the affinity of *Staphylococcus aureus* to nasal mucosal cells of carriers of this organism was significantly greater than that of noncarriers. In the present study, the adherence of *S pyogenes* to nasal mucosal cells from patients with chronic sinusitis was significantly greater than to those from normal subjects. These investigations point out the importance of bacterial adherence to epithelial cells as an essential factor for the establishment of infectious disease and suggest that the greater affinity of bacteria to mucosal cells of infection-prone patients is due to certain changes in the properties of mucosal cells and host environment rather than in the bacteria. Differences in the specificity or the distribution of host cell receptors, impairment of mucociliary clearance mechanism, and deficiency of specific immune defense are considered as host factors for the greater affinity of bacteria, but details are not known.

Secretory IgA is the chief agent in external secretions that protect the body against both infectious microorganisms and inert foreign macromolecules. Williams and Gibbons[4] demonstrated that secretory IgA isolated from parotid fluid specifically inhibited the adherence of *Streptococcus* strains to human buccal mucosal cells, although secretory IgA had no effect on strains against which secretory IgA had no antibody activity. It was suggested by this observation that secretory IgA inhibits the bacterial adherence to mucosal surfaces by attaching itself to the bacterial surface and interfering directly with the bacterial surface antigenic component's adherence to the epithelium. In a previous study,[5] we reported that the secretory IgA antibody activity specific for the M protein was significantly lower in patients with chronic sinusitis than in normal subjects although the secretory IgA content in nasal secretions was elevated in chronic sinusitis. In the present study, the adherence of *S pyogenes* was reduced in the group having secretory IgA antibody activity against the M protein, without correlating to the concentration of secretory IgA in nasal secretions. This investigation does not indicate the direct function of secretory IgA as reported by Williams and Gibbons,[4] because nasal mucosal cells were washed before being mixed with bacterial suspension. However, it can be speculated that secretory IgA possessing specific antibody activity still remains on the washed mucosal cells, or that the specific antibody activity of secretory IgA in nasal secretion may be related to the ability to prevent or block the bacterial adherence of the mucosal cells, although there is no scientific proof.

In conclusion, results of the present study show that the adherence of *S pyogenes* to nasal mucosal cells was greater in patients with chronic sinusitis than in normal subjects, and that it was enhanced in the group having no antibody activity of secretory IgA against the M protein. In addition to these findings, the specific antibody activity of secretory IgA is reduced in chronic sinusitis. This evidence demonstrates that mucosal immunity preventing or blocking adherence of bacteria to mucosal cells is impaired in this diseased mucosa.

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CONFERENCE ON THE EUSTACHIAN TUBE AND MIDDLE EAR DISEASES

The Conference on the Eustachian Tube and Middle Ear Diseases will be held October 26-29, 1989, in Geneva. For further information, contact Prof Jacob Sade, Chairman, c/o The Secretariat, Conference on the Eustachian Tube and Middle Ear Diseases, Intercontinental Hotel Geneva, 7-9 CH du Petit Saconnex, CH-1211 Geneva 19, Switzerland.

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EFFECT OF PROSTAGLANDIN E2 AND BACTERIAL ENDOTOXIN ON THE RATE OF DYE TRANSPORT IN THE EUSTACHIAN TUBE OF THE CHINCHILLA

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DAVID J. LIM, MD
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To determine the effects of various biologic agents on the rate of fluid transport in the eustachian tube of the chinchilla, we have established an in situ method of measuring dye transport in which the bulla remains intact. The normal rate of dye transport from an injection site in the superior bulla to the nasopharyngeal orifice of the eustachian tube was 130 ± 10 seconds. Inhibition of ciliary activity with the local anesthetic bupivacaine resulted in a saturable delay of transport (> 15 minutes), while exposure to the β-adrenergic stimulator isoproterenol caused a significant increase in transport rate (79 ± 7 seconds). Two inflammatory mediators commonly found in chronic middle ear effusions were also tested for their effect on fluid transport by the mucociliary apparatus. Bacterial endotoxin reduced transport rates at high concentrations, while prostaglandin E2 had no effect at any concentration tested.

KEY WORDS — endotoxin, eustachian tube, middle ear, mucociliary transport, prostaglandin E2.

INTRODUCTION

The middle ear is protected by the eustachian tube, including the action of the tubal muscles and the mucociliary apparatus, which are needed for this protective function. The tubal muscles are thought to be involved in pressure equilibration and aeration, and the mucociliary activity of the mucosal surface provides protection against invading organisms from the nasopharynx. Once a middle ear effusion is formed, clearance of fluid and debris from the middle ear space is mediated by both muscular action[1,2] and mucociliary transport.[3,4] Both systems serve to propel unwanted materials to the nasopharynx for elimination. It has been suggested that disruption of the mucociliary system leads to the development of fluid in the middle ear, and continued dysfunction of the mucociliary apparatus in the middle ear and eustachian tube may be responsible, in part, for the chronicity of these effusions.[5,6] Ciliary activity is, in fact, so important that the authors of a recent report[7] advocate using direct observation of this phenomenon to predict the future health of the middle ear in patients presenting with otitis media.

To assess the effect of various biologically active agents on the mucociliary transport system, it is necessary to establish the normal rate of fluid transport from the middle ear to the nasopharyngeal orifice of the eustachian tube. This study was undertaken to determine baseline transport rates using an in situ system in the chinchilla in which the bulla remains intact. In addition, this animal model was used to assess the effect on transport rates of two biologically active agents commonly found in middle ear effusions, prostaglandin E2 (PGE2) and bacterial endotoxin, to test the hypothesis that bacterial products or inflammatory mediators may affect the mucociliary transport mechanism, leading to chronicity of the effusion.

MATERIALS AND METHODS

Experimental Animals. Healthy adult chinchillas (*Chinchilla laniger*; 350 to 650 g), free of middle ear infection as determined by otoscopic examination, were used. A total of 84 ears (from 46 animals) were tested. Transport times from 20 ears were used to establish average baseline transport time (ten with dye alone; ten with 0.15 mL saline 15 minutes prior to dye injection). A minimum of five ears (range, five to 11) was used to establish transport time for all other agents tested. The chinchilla was chosen because this species is used extensively in studies of experimental otitis media, and the animal's large middle ear space, surrounded by an extremely thin bone, allows for dye injection directly into the cavity, leaving the tympanic membrane intact.

Operation. The chinchillas were anesthetized with a combination of xylazine (approximately 2 mg, intramuscularly; Rompun, Haver-Lockhart) and ketamine (approximately 13 mg, subcutaneously; Vetalar, Parke-Davis) prior to the incision to split the soft palate. When the openings to the eustachian tubes were clearly visible, the nasopharyngeal space was packed with saline-moistened gauze that was left in place until immediately before bulla injection.

From the Otological Research Laboratories, Department of Otolaryngology, Ohio State University College of Medicine, Columbus. Supported in part by National Institutes of Health/National Institute of Neurological and Communicative Diseases and Stroke grant NS 08854.

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EFFECT OF BIOLOGICALLY ACTIVE AGENTS ON RATE OF DYE TRANSPORT FROM MIDDLE EAR TO NASOPHARYNX OF CHINCHILLA

Agent	*Concentration*	*Transport Time** *(seconds ± SEM)*
Control	—	130 ± 10
Isoproterenol	0.005 %	79 ± 7†
Bupivacaine§	0.0005 %	163 ± 45
Salmonella typhimurium lipopolysaccharide	10 μg/mL	178 ± 44
	100 μg/mL	110 ± 21
	1,000 μg/mL	476 ± 47†
Prostaglandin E2	10^{-10} *M*	115 ± 7
	10^{-8} *M*	109 ± 13
	10^{-6} *M*	141 ± 29

*Each is mean of five or more replicates.

†Significantly different from control values ($p \leq .01$).

§At concentrations of 0.001 %, 0.005 %, and 0.5 %, majority of values exceeded 15-minute limit.

Bulla Injection. The anesthetized animals were placed in a prone position on a dissecting board with the jaws clamped open to allow visualization of the orifice with the operating microscope. At the appropriate time, 0.15 mL of either sterile saline, bupivacaine HCl (Marcaine HCl, Winthrop-Breon), isoproterenol HCl (Isuprel HCl, Winthrop-Breon), *Salmonella typhimurium* lipopolysaccharide (LPS, Sigma), or PGE2 (Sigma) was injected. All were diluted in sterile saline to the desired concentration. Once 0.15 mL was injected into the superior bulla via a tuberculin syringe, with a 25-gauge needle as a vent, all solutions except LPS were followed exactly 10 minutes later by an injection of 0.15 mL 5.0% Coomassie brilliant blue (w/v) in 0.01 *M* phosphate-buffered saline (PBS). The LPS solutions were allowed to remain in the middle ear for 1 hour before dye injection.

A stopwatch was used to determine transport time. The watch was started at the moment of dye injection and stopped at the first appearance of dye at the eustachian tube orifice in the nasopharynx. Transport rate was calculated by dividing the average transport time in seconds by 9.5 mm, which is the approximate length of the ciliated portion of the chinchilla middle ear mucosa.[8]

Statistical Analysis. Data were summarized by calculation of the arithmetic mean and standard error of the mean based on a minimum of five replicates per experimental trial. Observations were assumed to be approximately normally distributed, and Student's *t* test was used to determine the differences between means. We selected $p < .05$ as the level of significance for all analyses.

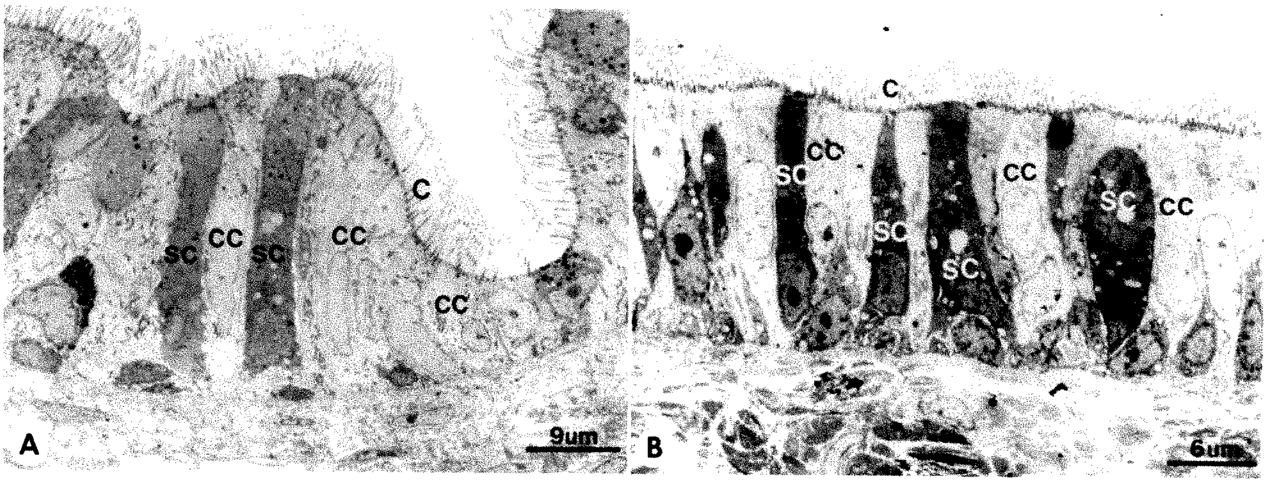
Light Microscopy. Selected eustachian tubes were processed for routine histologic examination. Following in situ fixation of the eustachian tube by injection of 2.5% glutaraldehyde in 0.05 *M* cacodylate buffer (pH 7.2 to 7.4) into the superior bulla, the tissue was postfixed in a 10% neutral buffered formalin, decalcified with 10% EDTA in 0.1 *M* Tris buffer (pH 6.95), dehydrated by passage through a graded series of ethanol washes, and embedded in glycol methacrylate (JB-4, Polysciences). Sections were cut and stained with hematoxylin and eosin.

Transmission Electron Microscopy. Following exposure to various agents, ten chinchilla eustachian tubes were prepared for transmission electron microscopy by local fixation with 2.5% glutaraldehyde injected into the superior bulla. After dissection of the eustachian tube from the temporal bone, the tissue was further fixed with 2.5% glutaraldehyde in cacodylate buffer (pH 7.2 to 7.4), postfixed with 1.3% osmic acid buffered with *s*-collidine, dehydrated by passage through a graded series of ethanol washes, and embedded in epoxy resin. Specimens were examined with a Phillips 300 transmission electron microscope.

RESULTS

Before initiating transport studies we assessed the effect of volume on transport to avoid either too large a volume, which would lead to passive streaming of dye down the eustachian tube, or too small a volume, which would result in stagnant pooling of the dye in the inferior aspect of the chinchilla bulla. Volumes of dye greater than 0.20 mL resulted in immediate appearance of dye at the eustachian tube orifice, while those 0.10 mL or less failed to appear within the 15-minute limit arbitrarily set for dye transport. A volume of 0.15 mL gave reproducible results and therefore was used throughout these experiments.

The normal transport rate of dye from the middle ear cavity to the nasopharyngeal orifice of the eustachian tubes (with or without an injection of saline 10 minutes prior to dye) was 130 ± 10 seconds (see Table). The dye was seen to move in a tonguelike front from the deeper aspects of the orifice, beginning as a faint grey-blue haze and building to a deeper blue as it began to accumulate. To assure ourselves that we were measuring transport of dye by ciliary activity, we attempted to manipulate the transport rates by accelerating or decelerating the ciliary beating with chemical agents. The β-adrenergic stimulator isoproterenol caused a significant increase in dye transport from an average of 130 seconds to 79 ± 7 seconds ($p \leq .01$) at a concentration of 0.005% (v/v). The local anesthetic bupivacaine had no effect on transport rates at a concentration of 0.0005%; however, at concentrations of 0.001%, 0.005%, and 0.5%, dye transport times were prolonged, with the majority of times in each category exceeding the 15-minute cutoff. *Salmonella typhimurium* endotoxin did not affect transport rates at either 10 or 100 μg/mL. At 1,000 μg/mL, however, average dye transport time was 476 ± 47 seconds, which was significantly



Transmission electron micrograph of chinchilla eustachian tube epithelium following exposure to A) 1,000 μg/mL lipopolysaccharide for 1 hour and B) 0.5% bupivacaine for 10 minutes. SC — secretory cell, C — cilia, CC — ciliated cell.

slower than with control injections or the two lower concentrations of LPS ($p \leq .005$). Prostaglandin E_2 had no effect on transport rates at any concentration tested (10^{-6}, 10^{-8}, 10^{-10} *M*).

To determine whether or not the delayed transport of dye noted after brief exposure to high concentrations of LPS or bupivacaine was due to either damage to or swelling of the eustachian tube epithelium rather than diminished mucociliary transport, we examined cross sections of this tissue by both light and transmission electron microscopy. Both light and transmission electron microscopic findings demonstrated that neither LPS nor bupivacaine instillation altered the morphologic integrity of the ciliated cells in the eustachian tube (see Figure), suggesting that the delayed dye transport by these agents was caused by reduced ciliary activity.

DISCUSSION

The mucociliary apparatus represents a critical defense system of the respiratory tract, including the middle ear, of both animals and humans. Through the action of the cilia, debris and fluid are moved from the middle ear space through the eustachian tube into the nasopharynx for elimination via the upper respiratory or digestive tract.[9] The first electron microscopic study of the middle ear mucosa of the guinea pig[10] demonstrated the presence of this system. The distribution of ciliated cells in the middle ear is very similar for humans,[11] guinea pigs,[12] and chinchillas.[8] Clearance of fluid from the middle ear via the eustachian tube was demonstrated by Compere in 1958.[3] Evidence that particle transport was also mediated by ciliary action was later offered by Sadé.[4] The suggestion that dysfunction of the mucociliary system could lead to the development of middle ear fluids[5] was demonstrated histologically by Lim and Klainer.[6]

Normal transport rates for a mucociliary system vary depending on the origin of the tissue, the particle being carried, and the method used to quantitate the movement, as well as the environmental conditions in which the assessment is made (ie, pH, humidity, temperature). Transport rates for frog palate epithelium, which has been extensively studied, vary from 2.1 to 3.2 mm/min for mucus-depleted tissue to 7.2 mm/min for undepleted tissue,[13] and most studies agree that approximately 20 mm/min is a maximum rate.[5,14,15] Rat trachea has been reported to have a similar transport rate of 10 to 15 mm/min.[16] In a study of the movement of tagged particles in the human nasal mucosa, average mucociliary flow rates of 7.5 mm/min were recorded.[17] Eustachian tube rates have been less thoroughly studied. Rogers et al[18] assessed tubal patency and function by injecting fluorescent dye into the middle ear and watching for its appearance in the nasopharynx. This occurred within 10 minutes in the absence of obstruction. The movement of dried India ink particles over the mucus blanket in the middle ear was tracked photographically by Sadé,[4] who found that the movement of these particles was independent of the direction in which the ear was tilted, that speed was inversely related to particle size, and that at times a maximum speed of 1 to 1.5 mm/s was attained. Inflamed mucosa was incapable of the same transport of particles, and some mucosae did not show clearance at all. Later studies by Sadé et al[19] and Brown et al[20] indicated the importance of mucous structure to middle ear clearance by the mucociliary system. While Ohashi et al[21] have shown that bacterial endotoxin will cause cessation of ciliary beating in all portions of the middle ear of the guinea pig, to our knowledge the factors that influence the rate of mucociliary transport of fluid from the middle ear cavity have not been systematically assessed in an animal model, particularly the chinchilla, which is the experimental animal most often used in otitis media research.

Baseline transport rates obtained in the animal model presented here indicate a calculated transport rate of approximately 4.3 mm/min, considering that the ciliated portion of the chinchilla middle ear mucosa (from the bony ridge to the nasopharyngeal orifice of the eustachian tube) is approximately 9.5 mm in length.[8] This value includes, by necessity, the time it takes the dye to diffuse from the injection site in the superior bulla to the inferior bulla, where it can pool and be picked up and moved by the ciliated epithelium; therefore, the calculation is likely to underestimate the actual rate slightly. This transport rate is well within the range of values reported for other mucociliary systems, and while not as sensitive as individual particle-tracking techniques, it offers the advantages of an intact bulla and a wholly in situ system sensitive enough to detect changes caused by biologic agents. Use of the local anesthetic bupivacaine HCl resulted in delayed transport of dye that was not due to ultrastructural damage to the eustachian tube. Bupivacaine is known to cause irreversible damage to ferret respiratory epithelium at a concentration of 0.13%,[22] and although we recorded no effect on transport at 0.0005%, there was significant inhibition at 0.001%, 0.005%, and 0.5%. Using a known β-adrenergic stimulator of rabbit oviduct cilia (isoproterenol),[23] we increased transport rates significantly.

While we cannot state with absolute certainty that the transport rates reflect ciliary activity exclusively, our ability to readily manipulate the rates with known ciliary accelerators and inhibitors was reassuring. In addition, the movement of the dye was often in a broad shimmering front from the eustachian tube into the nasopharynx, indicating an active transport rather than a passive outflow of fluid, which would have appeared as a narrow stream of dye. Since the animals were deeply anesthetized throughout the operative and experimental procedures and therefore did not swallow, thus eliminating any pumping function of the eustachian tube,[1] we believe that the tubal transport rates recorded were predominantly the result of mucociliary activity and not muscular activity. This is particularly convincing in light of the fact that the chinchilla eustachian tube has been shown to be continually patent.[24]

Middle ear fluids contain a variety of substances, including several biologic mediators of inflammation and LPS,[25,26] which are also known to have an effect on ciliary activity. Lipopolysaccharide or endotoxin is believed to contribute to the pathogenesis of otitis media with effusion. Indeed, endotoxin alone can lead to the presence of effusion in the middle ear of the chinchilla and causes capillary engorgement, edema, bleeding, and mononuclear cell infiltration.[27] Endotoxin from *Klebsiella pneumoniae* has been shown to result in a dose-dependent cessation of ciliary activity in all portions of the middle ear of the guinea pig.[21] In addition, endotoxin from type b *Hemophilus influenzae* was found to result in a variety of pathologic changes in the middle ear of guinea pigs that the authors attributed to transudation and injury to the mucosa that disturbed mucociliary transport activity.[28] Our data supported these findings by demonstrating that high concentrations of *S typhimurium* LPS, which is similar in many respects to that of *H influenzae*,[29] leads to extended transport rates.

Endotoxin levels of 0.5 to 512 ng/mL have been found in *H influenzae*-positive middle ear effusions.[26] In our model, ears received a total of 150 μg of LPS, which is in great excess of the levels cited above. However, we acknowledge that this model is unlikely to be sensitive enough to detect minor slowing of ciliary activity, and we do not know exactly how much of the endotoxin administered actually gains access to and acts upon tissues of the eustachian tube.

Prostaglandins are also frequently found in middle ear effusions in relatively high concentrations.[30-32] Prostaglandins E1 and E2 have been shown to cause marked increases in permeability and vasodilation-edema in dog middle ear and eustachian tube mucosa[33]; hence they may be one cause of middle ear effusion, and their continued presence may contribute to the chronicity of these effusions. The ability of PGE2 to elevate levels of lactate dehydrogenase, acid and alkaline phosphatase, calcium, and protein in the middle ear demonstrates a potential role for prostaglandins in the inflammation seen in otitis media.[34] Since PGE2 is also known to stimulate ciliary activity in rabbit oviduct[23] as well as sheep tracheal epithelial cell cultures,[35] we investigated its effect on transport rate in the chinchilla eustachian tube. That no effect was found over the range of concentrations tested may simply reflect the reduced hormonal sensitivity of this nonreproductive tissue, or it may reflect the fact that only minor changes, beneath the sensitivity threshold of this assay, were induced.

In conclusion, we have established a highly manipulatable and reproducible system for gross assessment of the rate of transport from the middle ear to the nasopharyngeal orifice of the eustachian tube in the chinchilla. This method offers several advantages over standard in vitro culture techniques, including the maintenance of an intact bulla and mucosa, which allows for better approximation of the in vivo situation. This method can be used to determine the effect of biologically active agents or bacterial products on transport rates. The fact that bacterial endotoxin has been shown to result in significantly delayed transport supports the hypothesis that nonviable bacteria or bacterial components that may be trapped in the tympanic cavity sustain the inflammation and contribute to the chronicity of the middle ear effusion.

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AUDITORY NERVE AND BRAIN STEM EVOKED RESPONSE THRESHOLDS IN INFANTS TREATED WITH GENTAMICIN AS NEONATES

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Thirty-two infants (18 full-term and 14 premature) who had been treated with gentamicin as neonates were examined to ascertain whether this drug induced hearing loss, even of low severity. Objective thresholds to clicks were obtained using auditory nerve and brain stem evoked responses. In addition, behavioral audiometry was performed. Serum concentrations before and after gentamicin treatment were at therapeutic levels. All infants were examined at least 1¼ months after cessation of therapy. Normal thresholds were obtained in all ears, with the exception of two with demonstrable middle ear effusion. It appears that gentamicin in therapeutic doses and serum concentrations, in the absence of renal insufficiency, does not cause hearing loss in neonates.

KEY WORDS — auditory behavioral responses, auditory nerve and brain stem evoked response thresholds, gentamicin, neonates.

INTRODUCTION

Aminoglycosides are used to treat suspected or proven bacterial infection in neonates, but renal, vestibular, and cochlear damage may result from this treatment. Even though hearing loss has been associated with gentamicin treatment in adults,[1-4] most studies of possible aminoglycoside ototoxicity in newborns, except for that of Bernard,[5] did not find a significant correlation between aminoglycoside antibiotics and hearing loss. In those few cases in which hearing loss was documented, factors other than the administration of these antibiotics may have caused the loss.[6]

With behavioral audiometry, Elfving et al[6] and Finitzo-Hieber et al[7] continued audiologic follow-up for a number of years until the children who received aminoglycosides as neonates were old enough to produce complete pure tone audiograms. Because some children were lost to follow-up, Marshall et al[8] and Finitzo-Hieber et al[9] recorded the auditory nerve and brain stem evoked response (ABR) of infants. However, as 30 to 40 dB hearing level (HL) was the lowest intensity stimulus presented, they were unable to detect mild or mild to moderate hearing losses. In addition, in the first ABR study mentioned,[8] the infants were examined too soon after the ototoxic drugs were administered, since it has been shown that hearing loss can progress for some time after treatment is discontinued.[2-4,10]

Our research was conducted to ascertain whether hearing loss even of low severity is induced by administration of aminoglycosides (gentamicin) in neonates. After a minimum period of 5 weeks after cessation of treatment, we tested infants with ABR thresholds to assess their hearing more accurately at a very young age.

MATERIALS AND METHODS

Subjects. With parental permission, 32 infants (64 ears) who had been treated with gentamicin during the first days of life were examined at the Speech and Hearing Center, Hadassah University Hospital. Of the 18 full-term infants (≥36 weeks' gestation), none were at risk for deafness. However, in seven of the 14 premature infants, there were additional risk factors such as low birth weight (≤1.5 kg) in three infants, low Apgar score (1 to 4 after 5 minutes) in another three, and hyperbilirubinemia (≥20 mg%) requiring exchange transfusion in one.

Gentamicin was administered to seven infants because of premature rupture of membranes and to five with transient tachypnea and suspected pneumonia; their blood cultures did not show bacterial growth. Neonatal sepsis was suspected in the remaining infants because of fever, recurrent apneas, constant acidosis, thrombocytopenia, or hypoglycemia. In this group, three had positive blood cultures (*Proteus mirabilis* [one], *Streptococcus faecalis* [one], and *Klebsiella pneumoniae* [one]) and three had positive urine cultures (*Escherichia coli* [two] and *Pseudomonas aeruginosa* [one]). Thus, six of 19 infants suspect for sepsis had positive cultures in normally sterile body fluids.

All infants were treated with gentamicin and ampicillin, except for one full-term neonate who received amikacin for 5 days after 5 days of gentamicin therapy.

Since gentamicin-related cochlear damage can

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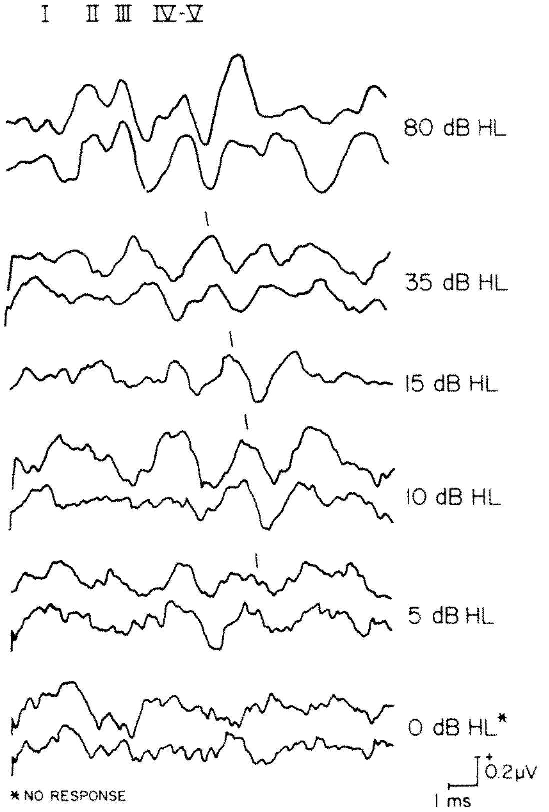

Fig 1. Example of auditory nerve and brain stem evoked response threshold testing in 2-month-old (corrected age) infant.

occur several weeks after treatment,[2-4,10] all the infants were tested audiologically not earlier than $1\frac{1}{4}$ months after completion of therapy. The chronologic ages of the test group were between 5 weeks and $5\frac{3}{4}$ months. As customary, we evaluated the results according to the corrected age, which was between full-term newborn and $5\frac{3}{4}$ months for our test group. ("Corrected age" means that premature infants were evaluated according to the norms for the age they would have been if they had been born full-term. For example, 8 weeks are subtracted from the chronologic age of an infant born at 32 weeks of gestation, so that if the infant is chronologically 12 weeks old, his/her performance is evaluated according to the norms for 4 weeks of age.)

Treatment Protocol. Neonates were treated with 2.5 mg/kg gentamicin, twice daily in the first week of life, and thrice daily in the second week, administered intravenously (IV) for 20 minutes each time. Gentamicin therapy was started between days 1 to 5 of life (1.81 ± 1.06, mean ± 1 SD), and administered for 3 to 10 days (5.47 ± 2.72). Serum concentrations were measured daily at approximately peak (half an hour after completion of IV administration) and trough (predose) times.

The average total dose (mean ± SEM) administered per patient was 29.45 ± 0.56 mg/kg (range, 15 to 67.5 mg/kg). Peak serum concentrations were 8.3 ± 0.06 μg/mL (range, 5.5 to 13.5 μg/mL). Trough levels were 1.39 ± 0.02 μg/mL (range, 0.5 to 2.7 μg/mL).

Hearing Evaluation. The hearing evaluation, performed at least 5 weeks after cessation of treatment, was based on ABR, infant behavioral audiometry, and, when necessary, tympanometry.

Behavioral audiometry was performed in a sound-proof room, with the infant sitting on the mother's lap. The stimulus used was free-field live voice at intensities of 45 to 70 dB HL.[11] Expected responses were awareness and startle behavior, in accordance with norms for the age tested.

The ABR was elicited by a 100-μs square wave (click) presented at alternating polarities at a repetition rate of 20/s. Clicks were presented through an earphone placed on the infant's ear. Each run numbered between 585 to 4,100 clicks, determined automatically according to the signal-to-noise ratio.

The response was recorded differentially between the vertex electrode and the electrode clipped to the earlobe of the test ear (silver disc electrodes, Grass Instruments), with the contralateral earlobe electrode serving as ground. The responses were filtered (200 to 2,000 Hz) and computer-averaged (C-ERA-100 electric response audiometer [Microshev]). Responses were displayed vertex-positive up.

The infants were examined in natural sleep or awake and relaxed, after feeding, and without sedation. At each intensity level, at least two tracings were obtained. At 120 dB SPL (80 dB HL) the latencies of waves I, II, III, and IV-V were measured (Fig 1). Stimulus intensity was then lowered until no waves could be identified in at least two trials of the same intensity. Response threshold was taken as the lowest intensity for which repeating responses were present. In addition, if the latency of wave I was prolonged above normal limits (1.60 ms) for adults, an otoscopic examination and/or tympanometry were performed.

RESULTS

All waves appeared at normal latencies in all but four infants examined. In these infants, the latency of wave I was prolonged (> 1.60 ms) in one ear only (normal latency in the other ear). This prolonged latency was due to middle ear effusion, as shown by flat curve tympanograms and confirmed by otoscopic examination. However, hearing loss as measured by elevated ABR thresholds was found in only two of these four infants, as the thresholds of the other two were within normal limits (20 dB HL). Of the remaining two, one infant had a mildly elevated ABR threshold in one ear, but in examinations conducted after the middle ear effusion was cleared, had normal hearing in that ear. The second infant had a moderate hearing loss in one ear, probably due to middle ear effusion, for which he was being

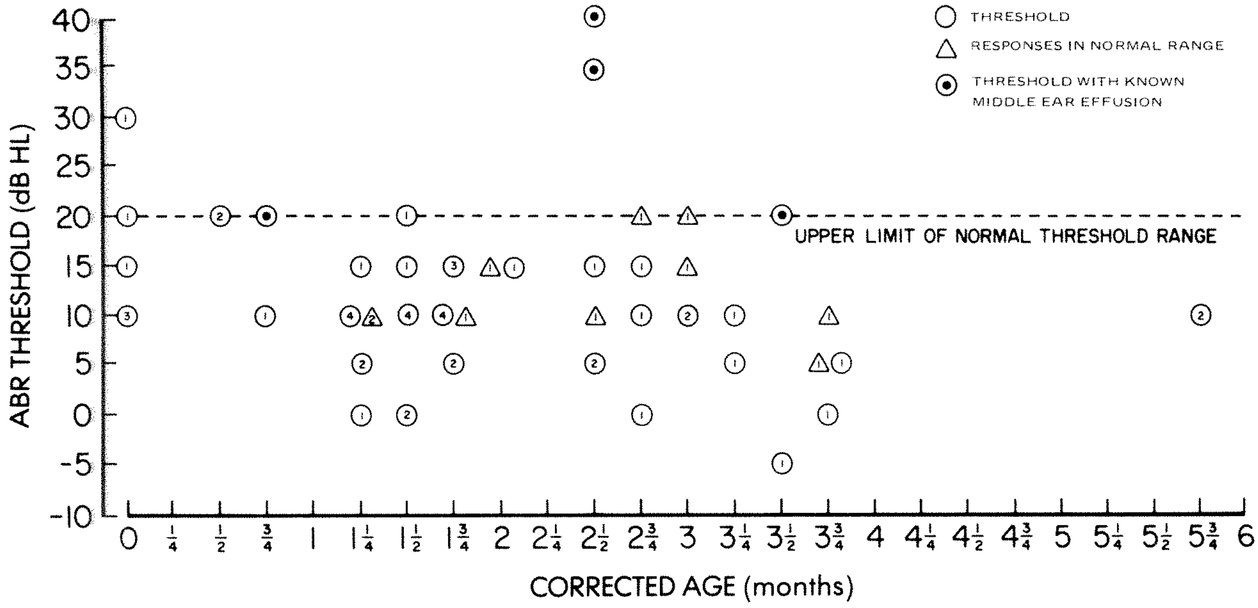

Fig 2. Results of auditory nerve and brain stem evoked response (ABR) threshold testing of 64 ears of infants treated with gentamicin as neonates. Arabic numerals within open circles and triangles refer to number of ears.

treated. However, he is now living in another city and did not return for follow-up. In all infants, interpeak latencies were appropriate for age.

Normal ABR thresholds in neonates are higher than in adults.[12] With maturation, thresholds are found within the adult range. In 51 ears of the infants tested after receiving gentamicin (29 infants), ABR thresholds were between −5 dB and 20 dB HL. Since at our clinic normal values for ABR thresholds in adults are −10 to 20 dB HL, the findings for these 51 ears were within the normal adult range. The remaining nine ears (seven infants) were tested until responses were seen in the normal range (minimum, 20 dB HL), but exact thresholds were unobtainable because these infants were awake and restless. In one premature infant tested at the gestational age of 41 weeks, thresholds were 20 dB HL in one ear and 30 dB HL in the other, considered normal for that age (Fig 2). In two infants, an elevated threshold was obtained in one ear, apparently due to middle ear effusion, as mentioned above.

All the free-field behavioral responses were normal for age. We observed a startle response to a live voice at 65 to 70 dB HL, depending on the subject's age, and speech awareness at 30 to 50 dB HL in infants over 3 months of age.

DISCUSSION

In 97% of the infants treated with gentamicin as neonates (62 of 64 ears), we found normal ABR thresholds, even when compared to adult norms. In the two cases of a unilaterally elevated threshold, there was demonstrable middle ear effusion.

By using ABR thresholds, it is possible to assess hearing status at least around 4 kHz accurately at an early age. As it is known that ototoxic drugs damage hearing first in the high frequency range, an additional advantage of the click ABR is its particular sensitivity to high frequencies around 4 kHz.[13,14] The behavioral tests examined speech-range frequencies, which are lower. Therefore, if normal ABR thresholds and appropriate-for-age behavioral responses were obtained, the possibility of hearing loss clearly seems negated. It is not possible to check responses to very high frequencies at this age, and we cannot be sure that there is no loss above 6 kHz.

Our findings do not agree with those of Bernard,[5] who reported that gentamicin causes hearing loss in neonates. Our results are similar to those of most other human and animal studies that showed that gentamicin and other aminoglycoside antibiotics given to neonates in controlled doses do not cause hearing loss.[6-9] These results are in contrast to the findings of Jackson and Arcierei,[1] Appel et al,[2] Winkel et al,[3] and Huizing et al[4] in adults treated with gentamicin, who subsequently developed hearing loss and damage to the hair cells in the inner ear. It seems that neonates are less susceptible to aminoglycosides than adults. Osako et al[15] showed this in rats at comparable developmental stages. Another possibility is that the adults studied received higher doses than the newborns and/or had higher serum concentrations and/or had renal insufficiency,[1-3,10] but comparison was difficult as the doses administered and the serum concentrations reached were not always stated. However, none of the newborns we examined suffered from renal insufficiency.

CONCLUSIONS

We tested a relatively small number of gentamicin-treated infants for exact ABR thresholds and found no evidence of hearing loss. In agreement with previously published data,[6-9] we found that when gentamicin is administered to neonates in recommended dosages in the absence of renal insufficiency, there is no reason to expect hearing damage; therefore, it is unnecessary to test their hearing beyond the screening usually conducted in well baby clinics.[16] However, should a neonate receive a larger dose of gentamicin and toxic blood levels are evident, referral for a thorough audiologic examination is mandatory within 3 to 6 months of age.

Therefore, where systematic screening of all infants and children of school age is conducted and the probability of undetected gentamicin-induced hearing loss is low, cost-benefit considerations lead us to conclude that ABR testing of all neonates who receive gentamicin is unnecessary.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY
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Ann Otol Rhinol Laryngol 98:1989

PROTEASE INHIBITORS IN MIDDLE EAR EFFUSIONS FROM EXPERIMENTAL OTITIS MEDIA WITH EFFUSION: KINETICS OF α1-ANTITRYPSIN AND α2-MACROGLOBULIN LEVELS

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Alpha 1-antitrypsin (α1-AT) and alpha 2-macroglobulin (α2-M) were measured by both immunochemical and functional assays in paired plasma and middle ear effusions (MEEs) from experimental otitis media models (serous otitis media [SOM], purulent otitis media [POM], and SOM + POM). The MEE/plasma ratio of α1-AT in SOM was significantly higher than that in POM ($p < .01$) because of the high α1-AT level in POM plasma. The ratio of both antitryptic activity and trypsin-binding activity in POM was significantly higher than that in SOM + POM ($p < .01$, $< .05$), and significantly lower than that in SOM ($p < .01$). The majority of both inhibitors in SOM exist as the free state, and the reaction between proteases and inhibitors in POM and SOM + POM is more active than that in SOM. It is concluded that both α1-AT and α2-M appear to play an important role in the protection of middle ear mucosa by forming protease-inhibitor complexes to reduce proteolytic damage in POM and SOM + POM models.

KEY WORDS — alpha 1-antitrypsin, alpha 2-macroglobulin, antitryptic activity, experimental otitis media, trypsin-binding activity.

INTRODUCTION

The induction of experimental otitis media with effusion (OME) by bacterial inoculation or eustachian tube obstruction (ETO) using chinchillas has been well established.[1-4] Both histological and biochemical studies using different types of animal OME models, namely, serous otitis media (SOM), purulent otitis media (POM), and the combination of SOM and POM (SOM + POM) may contribute to the better understanding of the inflammatory reactions in the middle ear cavity subsequent to bacterial infections and eustachian tube dysfunction.

Proteolytic degradation of the middle ear mucosa (MEM) by lysosomal proteases is one of the major factors inducing pathologic changes of the MEM. In recent years, the kinetics of the protease-inhibitor system and their roles in the pathogenesis of human OME have been investigated.[5,6] Alpha 1-antitrypsin (α1-AT) and alpha 2-macroglobulin (α2-M), which comprise about 90% of all the inhibitory function in plasma, and low molecular weight (LMW) inhibitors are known to regulate the proteolytic activity in the MEM.[7] Our previous studies also indicate that both α1-AT and LMW inhibitors have a significant role in the first-line defense mechanisms against proteolytic damage in normal chinchilla MEM.[8]

In this study, both immunochemical and functional assays of the major protease inhibitors, α1-AT and α2-M, were performed in middle ear effusions (MEEs) from three types of experimental OME models (SOM, POM, and SOM + POM). Proteolytic activity of nonspecific proteases and the presence of LMW inhibitors also were analyzed. The purpose of this study was to identify protease inhibitors (α1-AT, α2-M, and LMW inhibitor) and to measure nonspecific protease activity in order to understand the role of these inhibitors in the disease processes of various types of otitis media.

MATERIALS AND METHODS

MATERIALS

Serous Otitis Media Group. After anesthesia with ketamine HCl (20 mg/kg intramuscularly), the eustachian tubes of each animal were exposed operatively through the soft palate and were obstructed mechanically with Silastic sponges.[9] Fourteen animals were then allowed to develop MEE. Those animals forming effusions were identified by visual inspection of fluid through the tympanic membrane. When MEE was identified, each MEE was sampled via the epitympanic bulla and a blood sample was collected under anesthesia. Each MEE sample was cultured to verify the sterility of the effusion. Sampling was carried out up to 5 weeks. The samples were centrifuged to remove particulate materials and the clear fluid was frozen at −70°C until use.

Purulent Otitis Media Group. Animals were anesthetized with ketamine HCl and acute otitis

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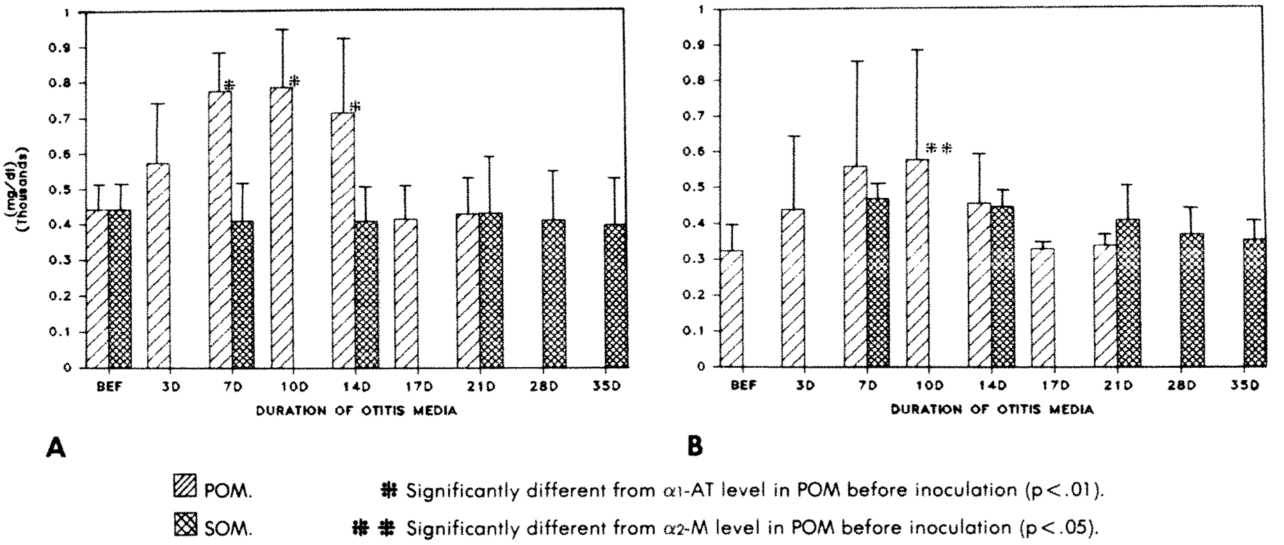

Fig 1. Concentration (mean ± SD) of A) α1-antitrypsin (α1-AT) and B) α2-macroglobulin (α2-M) in plasma. BEF — before.

media was induced by direct inoculation of type 7F *Streptococcus pneumoniae* (20 bacteria per ear) into each bulla. Three days after inoculation, 11 animals were anesthetized and an initial blood sample and MEE sample were taken. The MEE sample was cultured to verify that pneumococcal infection had occurred. Samples were taken from 3 days to 3 weeks after inoculation for comparative studies with the SOM group. All MEE samples were frozen after centrifugation until the time of assay.

SOM + POM Group. A combination of SOM and POM models was created by transbullar inoculation 2 weeks following the induction of SOM by ETO. A total of 128 MEE samples obtained 3 days to 5 weeks after induction of otitis media were analyzed: 50 with SOM, 46 with POM, and 32 with SOM + POM.

METHODS

Quantitation of α1-AT and α2-M Levels. A concentration of α1-AT in paired MEEs and plasma was measured by single radial immunodiffusion, and that of α2-M by electroimmunodiffusion, using specific antisera against chinchilla α1-AT and α2-M.[8] The value was expressed as the concentration ratio of MEE/plasma.

Antitryptic Activity (ATA) and Trypsin Binding Activity (TBA) in MEEs and Plasma. Both ATA and TBA in paired MEEs and plasma were measured by the same method described in a previous paper.[8] Both values also were expressed as the ratio of MEE/plasma.

Analysis of LMW Inhibitor in MEEs from SOM and POM. Pooled MEEs (1.0 mL) collected from both SOM and POM models were applied to Sephadex G-75 (Pharmacia Fine Chemicals, Uppsala, Sweden) and were equilibrated with 0.1 *M* sodium phosphate buffer (pH 7.4). Concentrations of α1-AT and α2-M, and ATA in each fraction, were measured.

Determination of Proteolytic Activity in MEEs. Proteolytic activity of nonspecific proteases in MEEs was measured by using a 0.1% heat-denatured bovine fibrinogen-agarose plate.[10] An MEE with a lytic zone was regarded as a positive reaction, and its activity was expressed as the lytic area (mm^2).

RESULTS

Concentration of α1-AT and α2-M in Plasma. Both α1-AT and α2-M levels in plasma from SOM and POM groups are shown in Fig 1. In SOM, the levels of both inhibitors did not change significantly from 1 to 5 weeks. In POM, α1-AT levels increased steadily after inoculation and reached their peak levels at 10 days after inoculation (782.8 ± 167.4 mg/dL). The levels of α1-AT at 7 and 10 days and 2 weeks after inoculation were significantly higher than those before inoculation ($p < .01$). At 3 weeks after inoculation, the α1-AT level returned to the normal preinoculation level (438.8 ± 72.2 mg/dL). The α2-M level also increased after inoculation, and its peak level was detected at 10 days after inoculation (575.3 ± 364.7 mg/dL) and was significantly higher than before inoculation ($p < .05$). At 3 weeks after inoculation, the α2-M level also returned to its preinoculation level.

Concentration Ratios (MEE/Plasma) of α1-AT and α2-M. The results of concentration ratios are summarized in Fig 2. In SOM, the ratio of α1-AT increased with the duration of ETO and reached the peak value at 3 weeks after ETO (1.81 ± 0.42) and was significantly higher than α1-AT ratios at 1 week ($p < .01$) and 2 weeks ($p < .05$) after ETO. A similar trend was observed in the ratio of α2-M, and its peak value was detected at 4 weeks after ETO (0.47 ± 0.13).

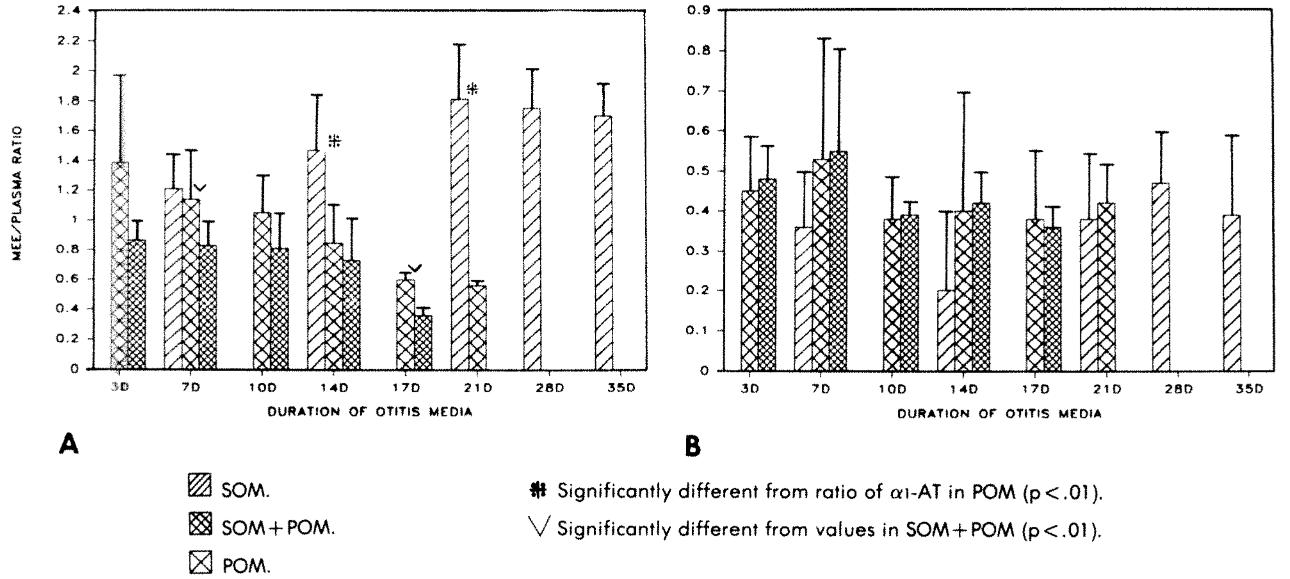

Fig 2. Concentration ratio (MEE/plasma) (mean ± SD) of A) α1-antitrypsin (α1-AT) and B) α2-macroglobulin in three types of OME models.

The ratio of α1-AT decreased with the duration of POM, and the peak value could be detected at 3 days after inoculation (1.39±0.56) and was significantly higher than α1-AT ratios at 2 weeks ($p<.025$), 17 days ($p<.01$), and 3 weeks ($p<.01$) after inoculation. The ratio of α2-M was relatively constant during the course of POM, and its highest ratio was detected at 1 week after inoculation (0.53±.30).

In SOM+POM, the ratio of α1-AT was under 1.0, and similar values (from 0.87 to 0.73) were detected from 3 days to 2 weeks after inoculation. A similar trend was observed in the ratio of α2-M, and the peak value was detected at 1 week after inoculation (0.55±0.24). Comparing the ratios of both inhibitors in the three groups, α1-AT in SOM showed the highest level and was significantly higher than that in the POM group ($p<.01$) at 2 and 3 weeks after induction of POM. The ratio of α1-AT in POM was significantly higher than that in SOM+POM ($p<.01$) at 7 and 17 days after bacterial inoculation.

Middle Ear Effusion/Plasma Ratio of ATA and TBA. In SOM, the ratio of ATA increased steadily with the duration of ETO, and its peak value was detected at 3 weeks after ETO (1.20±0.10) and was significantly higher than that at 1 week after ETO ($p<.01$) (Fig 3). The ratio of TBA increased gradually with the duration of ETO, and its highest level was detected at 5 weeks after ETO (0.47±0.13).

In POM, the kinetics of both ATA and TBA were quite different from those in SOM. The ratio of

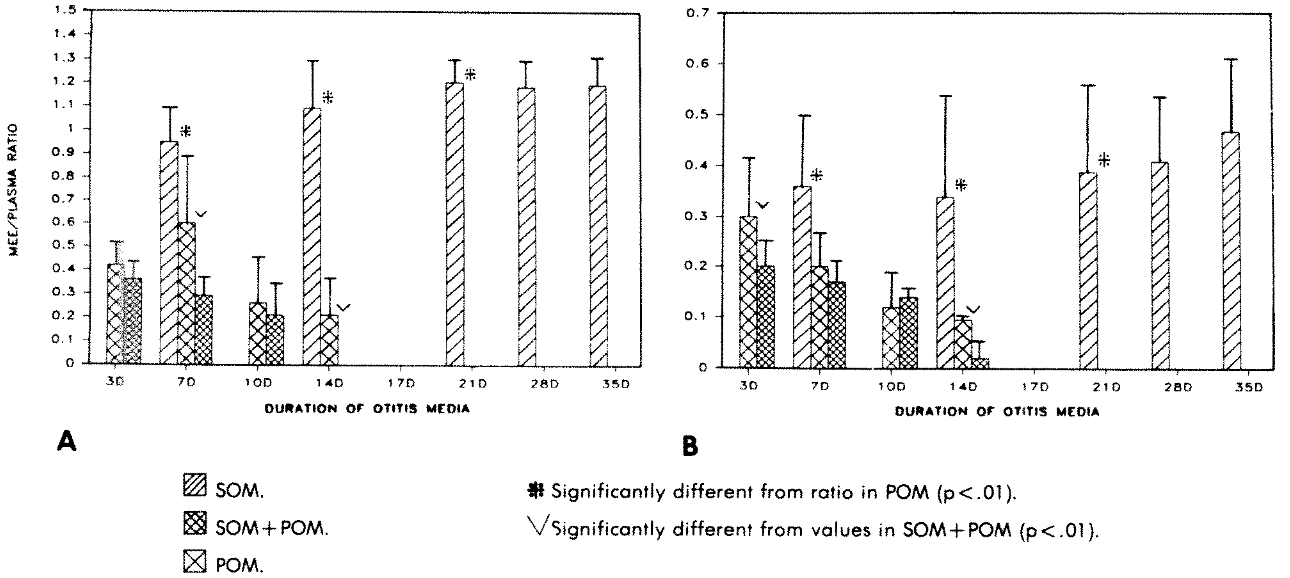

Fig 3. Middle ear effusion/plasma ratio (mean ± SD) of A) ATA and B) TBA in three types of OME models.

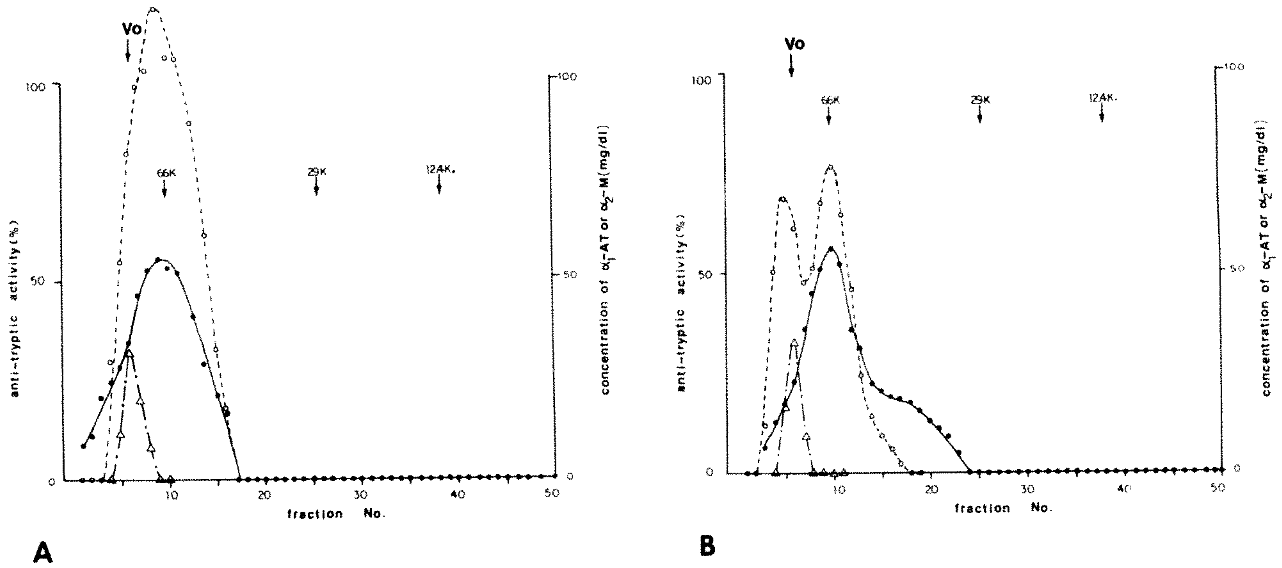

Fig 4. Elution profiles of Sephadex G-75 in pooled MEEs from SOM and POM groups. A) MEE (1 mL) diluted three times in SOM. B) MEE (1 mL) diluted three times in POM. Column size — K 15/30; buffer — 0.01 *M* sodium phosphate buffer, pH 7.4; flow rate — 1 mL/min; fraction size — 0.5 mL; ●——● — antitryptic activity (ATA, %); ○——○ — concentration of α1-antitrypsin (mg/dL); △—·—△ — concentration of α2-macroglobulin (mg/dL); Vo — void volume. Low molecular weight calibration kit for gel filtration was also applied (66 K, 29 K, 12.4 K).

ATA was the highest at 1 week after inoculation (0.60 ± 0.28) and was significantly higher than that at 10 days, 2 weeks, or 17 days after inoculation ($p < .01$). It decreased rapidly with the duration of POM, and no activity could be detected at 17 days after inoculation ($n = 4$). The ratio of TBA was the highest at 3 days after inoculation (0.30 ± 0.11) and was significantly higher than that at different days after inoculation. No TBA could be detected at 17 days after inoculation ($n = 4$).

In SOM + POM, the kinetics of both ATA and TBA were quite similar to those in POM. The ratio of ATA was the highest at 3 days after inoculation (0.36 ± 0.07) and was significantly higher than that at 10 days ($p < .05$), 2 weeks ($p < .01$), or 17 days ($p < .01$) after inoculation. No activity could be detected at either 2 weeks or 17 days after inoculation. The ratio of TBA was the highest at 3 days after inoculation (0.20 ± 0.05) and was significantly higher than that at 10 days ($p < .05$), 2 weeks ($p < .01$), or 17 days ($p < .01$) after inoculation. Comparing the ratios of ATA and TBA in the three groups, both ratios of ATA and TBA in POM were significantly higher than those in SOM + POM ($p < .01$, $p < .05$), and significantly lower than those in SOM ($p < .01$, $p < .01$) at 2 weeks after induction.

Relationship Between α1-AT Level and ATA, and Between α2-M Level and TBA. In plasma, there were significant correlations between the α1-AT level and ATA, and between the α2-M level and TBA, in all three groups ($p < .05$, $p < .01$ in SOM; $p < .05$, $p < .01$ in POM; and $p < .05$, $p < .05$ in SOM + POM). In MEEs, a significant correlation between the α1-AT level and ATA ($p < .05$) and between the α2-M level and TBA ($p < .01$) could be observed only in the SOM group.

Analysis of LMW Inhibitors in MEEs From SOM and POM Groups. Elution profiles of Sephadex G-75 in pooled MEEs from SOM and POM groups are shown in Fig 4. In both SOM and POM groups, apparent ATA could be detected in the fractions near void volume, which was identical to the single peak of the α1-AT level in SOM. In POM, two major peaks of the α1-AT level could be detected: one was in void volume and the other was identical to the single peak of the α1-AT level in SOM.

Proteolytic Activity in MEEs. By 0.1% fibrinogen-agarose plate assay, of which the detection limit was 10 μg/mL of trypsin (5 μL), MEE had no lytic zone in SOM, and only four of the 43 MEEs (10.9%; 24.2 ± 4.20 mm^2) in POM and nine of the 32 MEEs (28.1%; 45.3 ± 27.6 mm^2) in SOM + POM had a clear lytic zone.

DISCUSSION

Alpha 1-antitrypsin is a well-known acute phase reactant in humans, reflecting the degree of systemic inflammatory reaction. Both α1-AT and α2-M enter inflamed tissue from vessels via increased vascular permeability and control tissue protease activity, mainly from lysosomal proteases.[11] The levels of α1-AT and α2-M in plasma from the POM model indicated that both α1-AT and α2-M are acute phase reactants in the chinchilla, and pneumococcal infection in the middle ear could induce the systemic inflammatory reaction that peaks between 7 and 10 days after inoculation. About 3 weeks after inoculation, both inhibitor levels in plasma returned to the level before inoculation, which is identical to the time at which most POM animals had only a small amount of MEE. Juhn et al[12] and Hanson et al[13] have reported that the peak local inflammatory reaction in POM existed between 4 and 7 days after inoculation by analyzing lysozyme[12] and LDH[13] levels in MEEs. On the other hand, both inhibitor levels in plasma were constant during the course of SOM, suggesting that SOM is a more localized middle ear inflammation than POM.[14]

In SOM, the kinetics of α1-AT and α2-M ratios in MEEs were similar to those of ATA and TBA, and significant correlation was observed between α1-AT and ATA, and between α2-M and TBA. As both ATA and TBA reflect the level of free, unsaturated α1-AT and α2-M,[8,15] and the number of infiltrating leukocytes in MEEs is small in SOM,[12] it is obvious that the majority of both inhibitors in MEE from SOM exists as the free state, similar to that in human serous OME.[16] The chance of inhibitor inactivation by cleavage of an N-terminal peptide from α1-AT,[17] or saturation with proteases, seems remote during the course of SOM. Therefore, the levels of both inhibitors in MEEs of SOM are entirely dependent on the degree of the vascular permeability (VP) of the MEM, although secretory proteins from the mucosa of the eustachian tube may change the concentration of inhibitors. The VP of the MEM in SOM would reach its maximum level at about 3 weeks after ETO, based on the peak value of the MEE/ plasma ratio of α1-AT observed at 3 weeks after ETO.

In POM, the ratio of ATA was very low, compared to the α1-AT ratio in the same sample. Middle ear effusions in POM contain larger amounts (100 times) of leukocytes than those in SOM.[12] Alpha 1-antitrypsin combines with lysosomal proteases released from leukocytes to inhibit their activity. Some α1-AT may be inactivated by oxidants secreted from neutrophils,[18] and the complexed inhibitor is degraded and phagocytized by neutrophils and macrophages more rapidly than free inhibitor.[19] The decrease of the MEE/plasma ratio of α1-AT with the development of POM could be explained in that the α1-AT level in POM plasma increased after bacterial inoculation, and the degree of consumption and inactivation of α1-AT in MEE would surpass the degree of α1-AT leakage from MEM vessels. On the other hand, the α2-M ratio in POM was relatively constant compared to the α1-AT ratio. This may be due to the resistance of α2-M against proteolytic degradation and the dissociation of α2-M protease complexes to free α2-M.[20] The ratio of TBA decreased remarkably and lost its activity completely at 17 days after inoculation, suggesting that α2-M also combines with lysosomal proteases, especially α1-AT–resistant thiol proteases, to control their activity. It is obvious that the reaction between proteases and inhibitors is more active in POM than in SOM.

To measure proteolytic activity in MEEs, we used the micro–radial diffusion method, with a detection limit of 10 μg/mL of trypsin, sensitive enough for the detection of nonspecific proteases. This simple method is suitable for the detection of nonspecific protease activity in any biologic fluid.[10] That no proteolytic zone is found in most MEEs of POM (89%) suggests that the majority of protease activity in the supernatant MEEs of POM is controlled by inhibitors. Most of the POM animals (nine of 11) had no MEEs at about 3 weeks after inoculation and had residual ATA and TBA in MEEs until the last time of sampling. The presence of the residual inhibitory function in the MEEs could be an important factor in reducing mucosal damage by lysosomal proteases.

In SOM + POM, ratios of α1-AT and ATA were lower than those in POM alone, and 28% of the MEE samples had proteolytic activity. This indicates that protease inhibitors would be consumed more quickly in SOM + POM than in POM alone. Animals with SOM + POM and eustachian tube dysfunction cannot eliminate the inoculated bacteria or the inflammatory products from the middle ear, leading to a greater chance of mucosal damage by proteases. Two of the 11 POM and three of the five SOM + POM animals in which MEEs had no ATA and TBA at 17 days after inoculation have developed otitis media with persistent MEEs. It is very difficult to decide whether loss of the inhibitory function of protease inhibitors in MEEs is a cause or effect of the change from acute otitis media to the persistent stage. Histological study of the MEM in SOM, POM, and SOM + POM models has proven that pathologic changes of the MEM were the greatest in the SOM + POM model, and this model can proceed to chronic otitis media within a few months after inoculation.[21] Therefore, complete loss of inhibitory function in MEEs appears to be an important factor in inducing persistent mucosal damage.

The elution profile of Sephadex G-75 suggests that most α1-AT exists as the free form in SOM; and in POM, α1-AT consists of both α1-AT and α1-AT–protease complex. Low molecular weight trypsin inhibitors, which were detected in the middle ear bulla washings of normal chinchillas and were reported to have an important role in the first line of defense of the MEM,[8] could not be detected in MEEs of both SOM and POM groups. These inhibitors may have formed complexes with proteases, losing their inhibitory functions in the early stage of inflammation because of the limitation of supply and their high affinity to elastase and cathepsin G. Similar findings were observed in human acute otitis media.[22]

It is concluded that

1. Alpha 1-antitrypsin and α2-M are acute phase reactants in chinchillas, and the systemic inflammatory changes could be induced by POM, while SOM can be characterized as a more local-

ized inflammation without systemic change.

2. In SOM, MEE has a higher concentration of α1-AT than does plasma, and the majority of both inhibitors is present as the free state.
3. The reaction between proteases and inhibitors in POM is much more active than that in SOM, and is less active than that in SOM + POM.
4. Only a small amount of proteolytic activity was shown in MEE from SOM, while higher proteolytic activity was found in some of the MEEs from POM (10.9%) and SOM + POM groups (28%).
5. In POM and SOM + POM groups, inhibitory function of both α1-AT and α2-M plays an important role in the reduction of the mucosal damage by forming protease-inhibitor complexes.
6. Low molecular weight trypsin inhibitors in MEEs from both SOM and POM animals have little inhibitory function, suggesting that their consumption occurs in the early stage of middle ear inflammation.

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QUANTITATIVE BACTERIAL CULTURES AND BETA-LACTAMASE ACTIVITY IN CHRONIC SUPPURATIVE OTITIS MEDIA

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BETHESDA, MARYLAND

Aspiration of the exudate through open perforation was performed in 54 children with chronic suppurative otitis media. Eighty aerobic and 81 anaerobic isolates were recovered. Aerobic bacteria only were involved in 20 patients (37%), and anaerobic organisms only in seven (13%). Mixed aerobic and anaerobic isolates were recovered from 27 patients (50%). The most common bacteria isolated were anaerobic gram-positive cocci, *Bacteroides melaninogenicus* group, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. There were 45 β-lactamase-producing bacteria (BLPB), 30 aerobes, and 15 anaerobes recovered from 38 patients (70%). β-Lactamase activity was detected in 30 of the 38 ear aspirates (79%) that contained BLPB. All but one of these organisms were in excess of 10^4 colony-forming units/mL. The detection of β-lactamase activity in the ear aspirates provides evidence of the role of BLPB in the failure of penicillin therapy to eradicate chronic ear infection.

KEY WORDS — *Bacteroides* sp, β-lactamase–producing bacteria, chronic suppurative otitis media, microbiology.

INTRODUCTION

The polymicrobial aerobic/anaerobic nature of chronic suppurative otitis media has been established by several past and recent studies.[1-6] Although β-lactamase–producing bacteria (BLPB) were previously found in ear aspirates of children suffering from chronic otitis media,[1-6] the actual measurement of the enzyme activity in the ear aspirate was never determined.

This study investigated the actual activity of the β-lactamase enzyme in ear aspirates recovered from children with chronic otitis media and correlated the detection of this enzyme with the presence of aerobic and anaerobic BLPB in these aspirates.

PATIENTS AND METHODS

Patients. Middle ear exudates were aspirated from children suffering from chronic otitis media with perforation. Fifty-four children participated in this study. Patients' ages ranged from 6 to 17 years (average, 14 years 8 months). All were examined at the outpatient ear, nose, and throat clinics at Children's Hospital National Medical Center and the Washington Hospital Center in Washington, DC, because of exacerbation of the chronic otitis media. Patients had purulent drainage and a perforated tympanic membrane without tympanotomy tubes. The duration of the chronic otitis media ranged from 10 months to 8 years (average, 2.2 years). All patients had received previous courses of antimicrobial therapy for infection. The most recent course of antimicrobial therapy administered was ampicillin in 15 patients, penicillin V or G in 12, oxacillin or dicloxacillin in eight, a cephalosporin in ten, erythromycin in six, and trimethoprim-sulfamethoxazole in three. However, patients received no local, oral, or parenteral antimicrobial therapy for at least 1 month before sample collection.

Collection of Specimens. The external ear canal was cleaned of cerumen and pus with a blunt curette when indicated. It then was swabbed with povidone-iodine (Betadine) and a 70% alcohol solution and allowed to dry for 2 to 3 minutes. A culture of the external auditory canal was obtained using a swab soaked in sterile saline. The swab was spread immediately onto media supportive for the growth of aerobic and anaerobic bacteria in order to document the sterility of the external canal. The only cultures considered in the study were obtained from patients whose external ear canal culture showed no growth following the above procedure. In this manner, eight additional patients were excluded from the study. Five more patients, in whom insufficient amounts of middle ear fluid were obtained, also were excluded from the study.

Collection of the exudate was done through a perforation in the tympanic membrane using an 18-gauge Medicut (Sherwood Medical Instruments, St Louis), which consisted of an 18-gauge needle covered by a plastic cannula and attached to a 2-mL syringe.[7] The needle was bent to a 45° angle, and the cannula was slipped forward to cover the tip. When the tympanic membrane was approached via an otoscope, the cannula was retracted, the eardrum was entered through the perforation, and the exudate was collected. Thus, no contact by the needle tip was made with the speculum or the auditory canal, and pus was collected only from the middle ear.

Microbiology. Each middle ear aspirate was diluted 1:10 in prereduced thioglycolate broth (Difco

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From the Naval Medical Research Institute, Bethesda, Maryland. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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TABLE 1. CONCENTRATION OF ORGANISMS ISOLATED IN 54 SAMPLES OF MIDDLE EAR FLUID FROM CHRONICALLY INFECTED EARS

		Colony-Forming Units Per Milliliter			
	Total No. of Isolates	*10^2*	*10^3*	*10^4*	*10^5*
Aerobic and facultative organisms					
Gram-positive cocci					
Streptococcus pneumoniae	7	0	1	1	5
α-Hemolytic streptococci	8	3	3	2	0
Group A streptococci	2	0	2	0	0
Staphylococcus aureus	12 (12)	1 (1)	1 (1)	2 (2)	8 (8)
Staphylococcus epidermidis	2 (1)	1	1 (1)	0	0
Gram-negative cocci					
Branhamella catarrhalis	4 (2)	1	1	0	2 (2)
Gram-negative bacilli					
Hemophilus influenzae	5 (3)	0	0	1 (1)	4 (2)
Proteus sp	11	2	2	1	6
Pseudomonas aeruginosa	11 (5)	2	3	2 (2)	4 (3)
Klebsiella pneumoniae	10 (7)	1	1 (1)	1 (1)	7 (5)
Escherichia coli	2	0	1	1	0
Serratia marcescens	3	0	1	0	2
Citrobacter diversus	3	0	1	2	0
Subtotal	80 (30)	11 (1)	18 (3)	13 (6)	38 (20)
Anaerobic organisms					
Gram-positive cocci					
Peptococcus sp	15	2	3	2	8
Peptostreptococcus sp	10	2	1	4	3
Gram-positive rods					
Clostridium sp	5	0	1	0	4
Bifidobacterium sp	7	3	1	1	2
Propionibacterium acnes	3	2	1	0	0
Gram-negative rods					
Fusobacterium sp	8	3	0	3	2
Bacteroides sp	12	2	2	6	2
Bacteroides fragilis group	4 (4)	0	1 (1)	1 (1)	2 (2)
Bacteroides melaninogenicus group	13 (9)	3 (1)	2 (1)	1 (1)	7 (6)
Bacteroides oralis	4 (2)	0	1	0	3 (2)
Subtotal	81 (15)	17 (1)	13 (2)	18 (2)	33 (10)
Total	161 (45)	28 (2)	31 (5)	31 (8)	71 (30)

Nos. of β-lactamase-producing isolates are in parentheses.

Laboratories, Detroit). The suspension was shaken vigorously and used immediately for quantitative colony count by inoculation onto aerobic and anaerobic media. Sheep blood agar, chocolate agar, and MacConkey's agar plates were inoculated for aerobes. The plates were incubated at 37°C aerobically (MacConkey's) or under 5% CO_2 and examined at 24 and 48 hours. For anaerobes, the material was plated on prereduced vitamin K_1-enriched brucella blood agar and anaerobic blood agar plates containing kanamycin and vancomycin and then placed into enriched thioglycolate broth (containing hemin, sodium bicarbonate, and vitamin K_1).[8] The plates were incubated in anaerobic jars and examined at 48 and 96 hours. The thioglycolate broth was incubated for 14 days. Anaerobes were identified by techniques previously described.[8,9] Aerobic bacteria were identified by conventional methods.[10] β-Lactamase activity was determined on all isolates by the chromogenic cephalosporin analog 87/312 method.[11]

Demonstration of β-Lactamase in Ear Aspirates. One ear aspirate per patient was analyzed for β-lactamase. After removing a portion of the suspension of ear aspirate for culture, the supernatant was separated by centrifugation for 15 minutes at 20,000 times gravity. β-Lactamase activity of ear aspirate supernatant was assessed with Nitrocefin (Glaxo, Middlesex, England), which turns red after exposure to β-lactamase. The reaction mixture consisted of Nitrocefin dissolved in phosphate-buffered saline, pH 7.2, at a concentration of 500 μg/mL. Nitrocefin solution (10 μL) was added to 10 μL of β-lactamase solution (Difco Laboratories, Detroit) or to 10 μL of pus supernatant. Negative controls contained 10 μL of nitrocefin plus 10 μL of either undiluted enzyme-negative ear fluid supernatant or trypticase soy broth. A color change from yellow to red usually became apparent within 5 to 7 minutes. Color changes in some specimens developed more slowly, requiring up to an hour.

RESULTS

In 23 patients, aspirates were obtained from both ears, but only those from the right ears were included in the final analysis (Table 1). Microorga-

TABLE 2. MICROBIAL ISOLATES IN NINE PAIRS OF EAR ASPIRATES THAT SHOWED DISPARITY IN TYPES OF BACTERIAL ISOLATES BETWEEN RIGHT AND LEFT EARS

Pair	*Right Ear*	*Left Ear*
1	*Staphylococcus aureus*, *Branhamella catarrhalis*	*S aureus*
2	*S aureus*, α-hemolytic streptococci	*S aureus*, *B catarrhalis*
3	*Klebsiella pneumoniae*, *Serratia marcescens*	*K pneumoniae*, α-hemolytic streptococci
4	*Peptostreptococcus* sp, *Fusobacterium* sp	*Peptostreptococcus* sp, *Fusobacterium* sp, *S aureus*
5	*Peptococcus* sp, *Bifidobacterium* sp	*Peptostreptococcus* sp, *Bacteroides melaninogenicus*
6	*S aureus*, *Proteus mirabilis*, α-hemolytic streptococci, *Peptococcus* sp, *B melaninogenicus*	*P mirabilis*, α-hemolytic streptococci, *B melaninogenicus*
7	*Hemophilus influenzae*, *Streptococcus pneumoniae*, *Peptostreptococcus* sp, *Bacteroides oralis*, *B melaninogenicus*	*S pneumoniae*, *Peptostreptococcus* sp
8	*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Fusobacterium* sp	*S aureus*, *P aeruginosa*, *B melaninogenicus*
9	*P aeruginosa*, *Clostridium* sp, *Propionibacterium acnes*	*P aeruginosa*, *Clostridium* sp, *Peptostreptococcus* sp

nisms were recovered from all 54 patients included in the study. A total of 80 aerobic (1.5 per specimen) and 81 anaerobic isolates (1.5 per specimen) were recovered from middle ears of the patients. Aerobic bacteria only were isolated in 20 patients (37%), and anaerobic organisms only in seven patients (13%). Mixed aerobic and anaerobic isolates were recovered from 27 patients (50%). Thus, anaerobes were recovered from 63% and aerobes from 87% of the patients studied.

The most common bacteria isolated from the middle ear aspirates, in descending order of frequency, were anaerobic gram-positive cocci, *Bacteroides melaninogenicus* group, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.

In ten patients (19%; with eight aerobic and two anaerobic bacteria present) only one isolate was recovered. In the rest of the cases in which mixed anaerobic and aerobic bacteria were present, from two to six isolates per specimen (average, 3.7 isolates per specimen) were recovered. The combinations of the different isolates varied, and there was no consistent pattern. The colony counts exceeded 10^4 colony-forming units (CFU)/mL in 47 of the 54 cases (87%).

Differences in the microbiology of one ear versus the other were found in nine of the 23 patients (39%) from whom specimens from both ears were cultured (Table 2).

There were 45 BLPB (30 aerobes and 15 anaerobes) recovered from 38 patients (70%; 1.2 isolates/patient). These included all isolates of *S aureus* and *Bacteroides fragilis* group, nine of 13 of *B melaninogenicus* group, five of 11 of *P aeruginosa*, seven of ten of *K pneumoniae*, two of four of *Bacteroides oralis*, three of five of *Hemophilus influenzae*, two of four of *Branhamella catarrhalis*, and one of two of *Staphylococcus epidermidis*.

Most of the BLPB were recovered from patients recently treated with either a penicillin (27 patients) or a cephalosporin (five patients). β-Lactamase activity in the ear aspirates was detected in 30 of the 38 aspirates (79%) that contained BLPB (Table 3). The enzyme was detected in all but one of the aspirates that contained 10^4 CFU/mL or more, and it was not detected in six of the seven aspirates containing less than 10^4 CFU/mL. No β-lactamase activity was detected in the 16 aspirates that did not harbor BLPB.

The patients could be divided into three groups according to the types of BLPB recovered from their ear aspirates: 1) only aerobic BLPB recovered, 2) only anaerobic BLPB recovered, and 3) both aerobic and anaerobic BLPB recovered. No differences in the enzyme activity were found in the aspirates of patients with different *Bacteroides* groups. However, the color change was noted to be faster in the group of patients from whom aerobic

TABLE 3. β-LACTAMASE ACTIVITY IN RELATION TO PRESENCE OF β-LACTAMASE-PRODUCING BACTERIA IN EAR ASPIRATES

	Total No. of Patients	*No. of Patients in Whom β-Lactamase Activity Was Detected*
No BLPB recovered	16	—
Only aerobic BLPB recovered		
Staphylococcus aureus	7	6
Staphylococcus epidermidis	1	0
Branhamella catarrhalis	2	1
Hemophilus influenzae	3	3
Pseudomonas aeruginosa	3	3
Klebsiella pneumoniae	5	3
S aureus, *K pneumoniae*, *B catarrhalis*	2	2
Only anaerobic BLPB recovered		
Bacteroides sp	8	6
Aerobic and anaerobic BLPB recovered		
S aureus, *Bacteroides* sp	3	3
P aeruginosa, *Bacteroides* sp	2	2
K pneumoniae, *Bacteroides* sp	2	1
Total	54	30

BLPB — β-lactamase-producing bacteria.

bacteria were recovered than in those from whom only *Bacteroides* sp were recovered.

DISCUSSION

We were able to recover anaerobic bacteria in over half of our patients, a finding that confirms previous data.[1-6] We were also able to demonstrate the presence of BLPB in more than two thirds of our patients. The recovery of BLPB is not surprising, since most of our patients received multiple courses of antimicrobial agents including penicillins, which might have selected for BLPB. However, the ability to detect the actual enzyme activity in the ear aspirate raises the possibility that this enzyme not only is able to protect the BLPB but also can degrade penicillin in the ear fluid, thus protecting penicillin-sensitive organisms.

Many microorganisms, including *Bacteroides* sp, produce β-lactamase, which enables them to resist penicillin.[12] Until recently, most *B melaninogenicus* and *B oralis* strains were considered susceptible to penicillin. However, within the past decade, penicillin-resistant strains have been reported with increasing frequency.[13]

The appearance of penicillin resistance among *Bacteroides* has important implications for chemotherapy. Many penicillin-resistant bacteria can produce enzymes that degrade penicillins or cephalosporins. Such organisms, when present in a localized soft-tissue infection, can release the enzyme into the environment. By so doing, they degrade penicillin in the area of infection, and protect not only themselves but also penicillin-sensitive pathogens. Therefore, penicillin therapy directed against a susceptible pathogen might be rendered ineffective by the presence of a BLPB.

β-Lactamase–producing organisms may "shield" penicillin-susceptible organisms from the activity of penicillin, thereby contributing to their persistence. The ability of BLPB to protect penicillin-sensitive microorganisms has been demonstrated in vitro and in vivo.[14-16] This phenomenon also has been observed in clinical infections. De Louvois and Hurley[17] demonstrated degradation of penicillin, ampicillin, and cephaloridine by purulent exudates obtained from abscesses. Studies by Masuda and Tomioka[18] demonstrated possible β-lactamase activity in empyema fluid. Bryant et al[19] studied the β-lactamase activity in samples of pus obtained from 12 patients with polymicrobial intra-abdominal abscesses or polymicrobial empyema. The supernatant of six specimens was also able to rapidly inactivate penicillins and cephalosporins.

Recently, we tested the hypothesis that β-lactamase–producing strains of *B fragilis* and *B melaninogenicus* can protect streptococci from penicillin in mice.[20] A mixed infection was induced in mice in the form of a subcutaneous abscess involving a penicillin-susceptible streptococcus and a β-lactamase–producing strain of either *B melaninogenicus* or *B fragilis*.

The infected animals were treated with parenteral penicillin, penicillin and clavulanic acid, or clindamycin. All modes of treatment prevented the formation of abscesses in animals inoculated with streptococci alone, but not in those inoculated with mixed streptococci and *Bacteroides*. However, there was marked reduction in the size of the abscesses and in the total number of viable streptococci in the pus from animals treated with penicillin and clavulanic acid or clindamycin, compared with nontreated controls and with those treated with penicillin alone. Clindamycin or the combination of penicillin and clavulanic acid, which are active against both streptococci and *Bacteroides*, was most effective.

The isolation of BLPB from over two thirds of chronically inflamed ears and the ability to actually measure the activity of the enzyme in the ear aspirate raise the questions of whether the treatment of chronic otitis media with penicillins is adequate in all instances, and whether therapy should be directed at the eradication of these organisms whenever they are present. Further studies of this problem are under way. The discrepancies in microbiology between the two ears in more than a third of the patients in whom both ears were cultured highlights the need to obtain specimens from both infected ears. This is of particular importance when consideration is given to selection of proper antimicrobial agents. It should be remembered that in addition to antimicrobial therapy, surgical drainage and correction of underlying disease are critical adjunctive aspects of treatment. As more data from in vitro, animal, and patient studies accumulate, logical approaches should be found for the eradication of chronic ear infections.

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FLEXIBLE TRANSBRONCHIAL NEEDLE ASPIRATION FOR THE DIAGNOSIS OF SARCOIDOSIS

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To determine the value of transbronchial needle aspiration biopsy in the diagnosis of sarcoidosis, we reviewed a 1-year experience of consecutive patients with sarcoidosis presenting with hilar and/or paratracheal adenopathy. The sensitivity of transbronchial needle aspiration biopsy in obtaining specimens of noncaseating granulomas was 90%. This yield exceeds that of most published reports of transbronchial lung biopsy and bronchial mucosal biopsy and suggests that transbronchial needle aspiration biopsy may be a valuable diagnostic tool in the evaluation of these forms of sarcoidosis.

KEY WORDS — fiberoptic bronchoscopy, sarcoidosis, transbronchial needle aspiration biopsy.

INTRODUCTION

Sarcoidosis most commonly presents with nonspecific clinical symptoms, a normal physical examination, and a chest radiographic pattern that may be highly suggestive (class I), or less so (class II or III). The diagnosis is confirmed by obtaining histologic evidence of noncaseating granulomas in the lung, lymph nodes, skin, or other tissue. When less invasive tissue samples are unrewarding, the accepted procedure of choice is the transbronchoscopic lung biopsy.[1] The most benign form (class I) is associated unfortunately with a positive transbronchial lung biopsy in only 60% of cases, while class II patients have approximately an 88% positive yield on biopsy.[1-3] To evaluate the role of transbronchial needle aspiration (TBNA) biopsy in the diagnosis of sarcoidosis presenting with hilar and/or mediastinal adenopathy, we reviewed a 1-year experience of consecutive patients presenting with mediastinal and hilar lymphadenopathy.

MATERIALS AND METHODS

Between May 1985 and April 1986, 61 patients were referred to the Johns Hopkins Bronchoscopy Service for evaluation of an abnormal chest radiograph with hilar and/or mediastinal lymphadenopathy. The records of this consecutive series of patients were reviewed for final diagnosis, and diagnostic procedures performed were collated.

Patients were defined as having sarcoidosis if they had a compatible clinical history, physical examination, and chest radiograph and histologic evidence of noncaseating granulomas from tissue biopsy. Patients were defined as having diseases other than sarcoidosis if they had a definitive diagnosis based on histologic evidence of another disease.

Sarcoidosis was categorized by radiographic findings as class I (hilar and/or paratracheal adenopathy without parenchymal disease) or class II (hilar and/or paratracheal adenopathy with parenchymal disease). Computed tomography was performed on all patients, but classification was based on conventional radiography.

Informed consent was obtained from all patients, who then underwent fiberoptic bronchoscopy in the supine position. The transnasal approach, a standard 2-mm channel bronchoscope (Fujinon Y2S), and local anesthesia were used. Transbronchial lung biopsies (TBLBs) and bronchial mucosal biopsies (BMBs) were performed with standard forceps. Transbronchial needle aspirations were performed with an 18-gauge needle (Mill Rose Corp, Mentor, Ohio). The technique has been described previously.[4] One to three samples were attempted at each site, with the tissue analyzed for both histology and cytology. No effort was made to look at the adequacy of or yield from each biopsy attempt. Instead, all biopsy specimens from a single site were used as a combined specimen.

A positive biopsy for sarcoidosis was defined as noncaseating epithelioid granulomas in the absence of an identifiable foreign body, allergic tissue reaction, acid-fast bacillus cultures or stains, or fungal organisms. A biopsy specimen was considered adequate for evaluation as a TBLB if alveolar tissue was present, for BMB if bronchial mucosa was present, and for TBNA of lymph node if material consistent with the architecture of lymph nodes was present.

RESULTS

Of the 61 patients evaluated, 41 were proven to have a diagnosis other than sarcoidosis. These included lung cancer (26 patients), metastatic cancer (four patients), hematologic malignancy (three patients), and benign disorders (eight patients). Of the 20 patients diagnosed as having sarcoidosis, 14 were

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TABLE 1. COMPARISON OF TECHNIQUES FOR DIAGNOSING SARCOIDOSIS

Biopsy Method	*No. of Biopsies Performed*	*Positive Biopsy*	
		No.	*%*
TBLB	9	5	55.6
BMB	8	3	37.5
TBNA			
Right paratracheal	14	11	78.1
Left hilar	8	8	100.0
Right hilar	1	1	100.0
Carinal	3	3	100.0
Total	26	23	88.5

TBLB — transbronchial lung biopsy, BMB — bronchial mucosal biopsy, TBNA — transbronchial needle aspiration.

females. Twelve cases were class I, and the remainder class II. One patient has an initial nondiagnostic bronchoscopy (TBNA), but on repeat bronchoscopy, tissue consistent with sarcoidosis was obtained from all sites (TBLB, TBNA, and BMB). Another patient's biopsy showed "epithelioid histocytes and asteroid bodies." This patient was considered to have negative TBNA results.

The diagnostic biopsies from each site in the patients with sarcoidosis are summarized in Table 1. Transbronchial needle aspiration was performed in 26 sites, and adequate tissue specimens were obtained in 24 (92.3%). Eight of nine TBLB specimens (88.9%) were considered adequate. Eight of eight BMB specimens (100%) were considered adequate.

The diagnostic rates for TBNA, TBLB, and BMB sites were 88.5%, 55.6%, and 37.5%, respectively (Table 1). Both 1) TBNA and 2) TBLB, BMB, or both were diagnostic in 33.3% of patients in whom they were performed (Table 2). Six patients were diagnosed exclusively by TBNA when used in conjunction with TBLB and/or BMB. One patient was TBNA-negative, but positive with one of the other two procedures. Eight patients had only TBNA performed, and the procedure yielded diagnostic tissue in all. Eighteen of 20 patients (90%) had one or more positive TBNAs.

Cytologic examination of all specimens also was carried out, but was found to be diagnostic in only two of 20 patients.

There were no significant complications of the procedures. Minimal bleeding was noted at some of the biopsy sites, and none required treatment.

DISCUSSION

The diagnostic value of a test for any disease is dependent on the sensitivity and specificity of the test, the known probability that the patient has the disease, and the risk-benefit ratio of performing the test. In screening large populations for a disease, high sensitivity in a test takes precedence over specificity, while in testing an individual patient in whom one diagnosis is highly suspected, specificity becomes an important factor. Since the presence of noncaseating granulomas in the appropriate clinical setting is already highly specific for sarcoidosis, sensitivity and a low risk-benefit ratio become the most attractive features of a test for use in a patient in whom sarcoidosis is strongly suspected.

TABLE 2. SUCCESS OF CUMULATIVE DIAGNOSTIC STUDIES FOR SARCOIDOSIS

Biopsy Method	*Ratio of Positive Diagnoses/Pts*	*Diagnostic Rate (%)*
TBNA+, FB+	4/12	33.3
TBNA+, FB−	6/12	50.0
TBNA−, FB+	1/12	8.3
TBNA−, FB−	1/12	8.3
TBNA+	8/ 8	100.0
Total TBNA	18/20	90.0

TBNA — transbronchial needle aspiration, FB — forceps biopsy for transbronchial lung biopsy and/or bronchial mucosal biopsy, plus — positive biopsy, minus — negative biopsy.

Not all patients in our study had a TBNA, a TBLB, and/or a BMB, so we are unable to compare the sensitivity of these tests directly. However, we can compare the sensitivity of TBNA in the present study with the sensitivity of TBLB and BMB as published by others. In class I and II sarcoidosis, the sensitivity of the TBLB is 44%, 55%, 66%, and 85% (average, 62.5%) and 73%, 88%, 96%, and 100% (average, 89.3%), respectively.[1-3] The specificity nears 100%, with only the occasional misdiagnosed case of tuberculosis to detract from a perfect score. The sensitivity of BMB is reported to be 19% to 63%.[5-9] The specificity is unknown, but is presumed to be high.

In the present study, 18 of 20 consecutive patients had a positive TBNA, yielding a 90% sensitivity. The high sensitivity of TBNA should not be surprising, for it is likely that the density of granulomas is greater in lymph nodes than in lung tissue, especially in the milder forms of the disease. Of note is the fact that 83% (seven of eight) of class I patients had a positive TBNA. This contrasts with the reported 62% yield of TBLB in class I patients. The specificity of the TBNA is presumed to be the same as that of mediastinoscopy, which approximates 100%.[10,11]

All three methods — TBLB, BMB, and TBNA — have a low risk-benefit ratio. The reported complication rate from TBLB approximates 2% to 5%, with most being minor complications.[1] The complication rate from BMB is not quantitated, but is presumed to be low. Similarly, the complication rate from TBNA is undefined, but is thought to be less than 1%. In the present study there were no complications of TBNA.

Considering the above factors, TBNA appears to be an attractive addition to the diagnostic armamentarium of the endoscopist pursuing the diagnosis of sarcoidosis in patients presenting with hilar and paratracheal adenopathy.

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The University of Iowa Department of Otolaryngology–Head and Neck Surgery and the International Cochlear Implant Association will hold the Second International Cochlear Implant Symposium in Iowa City on June 4-9, 1990. The symposium will focus on basic science aspects of electrical stimulation on the auditory system, new cochlear implant strategies, clinical application in both adults and children, and rehabilitation and educational issues. Please submit abstracts to Bruce J. Gantz, MD, Department of Otolaryngology–Head and Neck Surgery, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

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NIH THERAPEUTIC STUDY OF FOLLICULAR THYROID CARCINOMA

The National Institutes of Health request the cooperation of physicians in referring patients with pure follicular thyroid cancer for a randomized clinical trial that will investigate the effectiveness of radiosensitizing agents in enhancing radioiodine therapy. Patients should have had primary thyroid surgery, without additional therapy for recurrences, and meet one of the following criteria: 1) age 40 years or older at the time of diagnosis with either primary tumor greater than 3 cm, regional lymph node metastases, extensive tumor capsule penetration, or extensive angioinvasion; or 2) any age with either tumor penetration into surrounding tissues or metastases outside of the neck.

All medical care expenses at NIH will be covered by the government except for transportation costs. Physicians will be informed regularly about the status of their patients and will continue to provide long-term care for them. Please write or telephone Dr Kenneth B. Ain or Dr Jacob Robbins, Bldg 10, Rm 8N315, National Institutes of Health, Bethesda, MD 20892; (301) 496-2831 or (301) 496-5761.

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ACUTE EFFECTS OF SULFUR DIOXIDE EXPOSURE ON THE MIDDLE EAR MUCOSA

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A variety of atmospheric pollutants are known to depress mucociliary function in the respiratory system. Since the mucociliary function in the middle ear is similar, and the middle ear may be invaded by atmospheric pollutants, we decided to investigate the possible contribution of sulfur dioxide to middle ear effusion. Guinea pigs were exposed for 24 hours to 300 ppm of sulfur dioxide or air. Immediately after exposure, ciliary activity and epithelial structure were examined close to the tympanic orifice (proximal site) and more distal to it (distal site). In the animals exposed to sulfur dioxide, no effusion was found in the tympanic cavity. Ciliary activity was reduced only in the distal site. Electron microscopy demonstrated hypersecretion in the proximal site and severe pathologic changes in the distal site. Although the normally functioning cilia in the proximal site may prevent retention of surplus secretions in the ear, sulfur dioxide may promote middle ear effusion when combined with other detrimental factors, because it stimulates mucus secretion in the proximal site and impairs ciliary function in the distal site.

KEY WORDS — ciliary activity, electron microscopy, middle ear, sulfur dioxide.

INTRODUCTION

Air pollution is a serious problem in big cities. A variety of atmospheric pollutants have been shown to exert an irritant effect on the respiratory mucosa that for most irritants is characterized by a depression of mucociliary function. The vulnerability of the mucociliary apparatus in the respiratory mucosa to exposure to irritant gases was demonstrated over 55 years ago.[1] Since then, evidence has accumulated on the pathologic effects of atmospheric pollutants on both in vitro and in vivo preparations. Short-term exposure (a few weeks) to high concentrations of sulfur dioxide causes squamous metaplasia, destruction of cilia, and goblet cell metaplasia in intermediate-size and small bronchi with accumulation of mucus in various animal species.[2-4] In 1956, Dalhamn[5,6] demonstrated ciliostatic effects in the tracheas of rats exposed to sulfur dioxide at a concentration of 10 ppm, and these findings were later confirmed by other investigators showing the detrimental effects of short-term exposure on mucociliary activity both in vitro and in vivo.[7,8] In healthy nonsmokers, short-term exposure to 10 ppm of sulfur dioxide causes transient impairment of nasal[9] and tracheobronchial[10] clearance.

A number of ultrastructural investigations have established that the lining of the middle ear has a mucociliary system similar to that of respiratory organs.[11-13] Since the middle ear mucosa could be invaded through the eustachian tube by noxious gases to which the respiratory mucociliary function is vulnerable, the mucociliary function in the middle ear might be expected to be similarly affected by atmospheric pollutants including sulfur dioxide. The purpose of this study is to elucidate experimentally the hitherto unknown influence of sulfur dioxide exposure on the middle ear mucociliary system.

MATERIALS AND METHODS

We used 20 male Hartley strain guinea pigs (average weight, 250 g) free of middle ear diseases as demonstrated by otoscopic examination. Ten animals were exposed for 24 successive hours to 300 ppm of sulfur dioxide in an environmental chamber. The remaining ten animals served as controls: they were exposed to gas containing air alone for 24 successive hours in the same kind of chamber. They were decapitated painlessly immediately after the exposure period, and middle ear mucosal samples were obtained from two different locations in each animal, because the nature of the middle ear mucosa varies considerably according to location.[11-13] The two locations chosen in this study were 1) close to the tympanic orifice (proximal site) and 2) more distal to the orifice (distal site). The details of these locations have been described elsewhere.[14,15]

Samples from the right middle ear were used for assessment of ciliary activity (beats per minute), and those from the left middle ear for electron microscopy. The ciliary activity in the most active cell of each sample was examined in a physiologic saline solution at an ambient temperature of 30°C by use of the direct and quantitative photoelectric method of Ohashi and Nakai.[16,17] The ciliary activity in every sample was determined five times, and average values were used. About 20 minutes was re-

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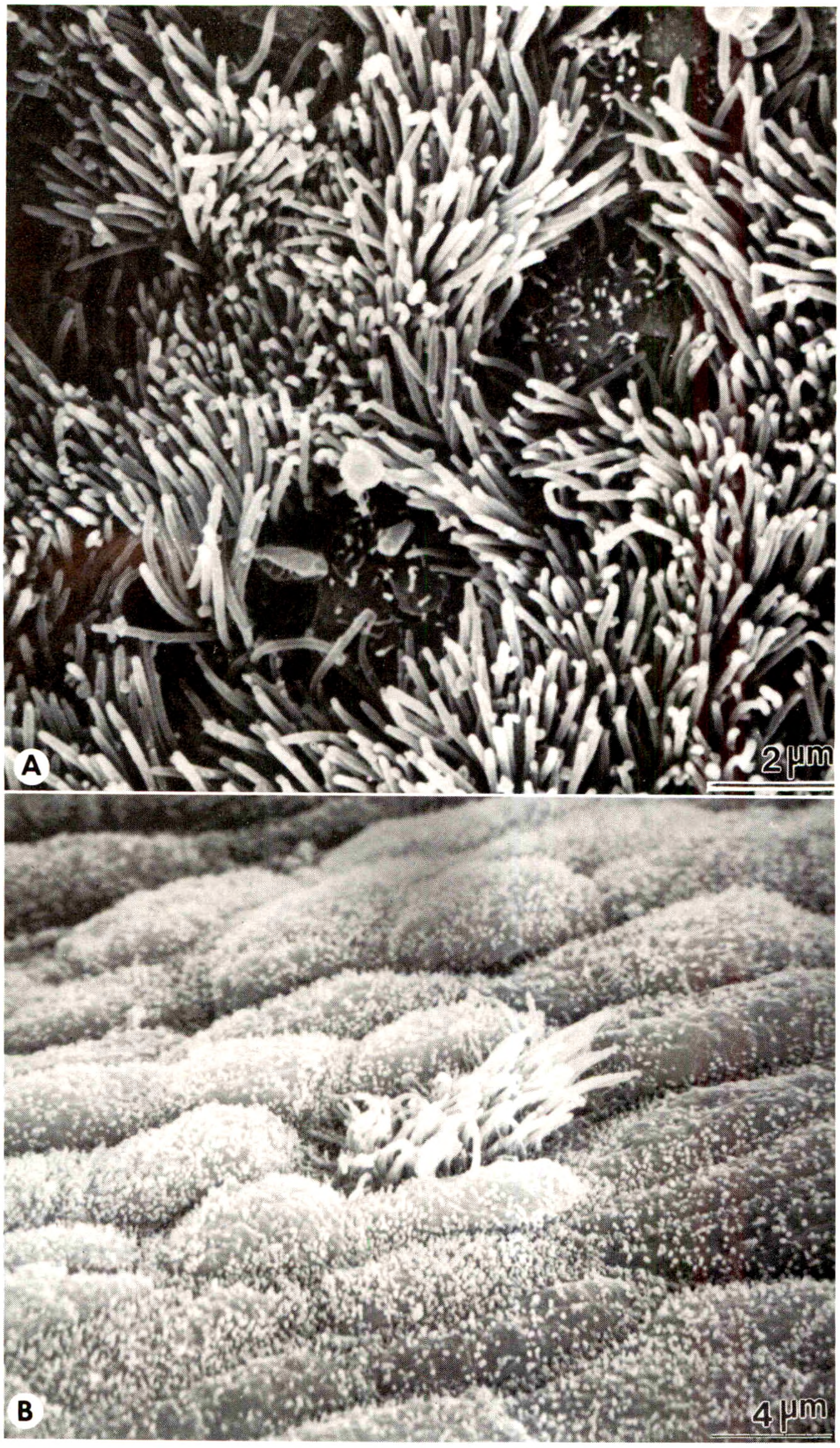

Fig 1. Scanning electron photomicrographs of middle ear mucosa of control animal. A) Proximal site (original x3,500). Abundant cilia with normal metachronal waves are seen, along with sporadic goblet cells between ciliated cells. B) Distal site (original x2,500). Sporadic ciliated cells are seen between nonciliated cells. No abnormal findings are observed.

quired to examine the ciliary activity in each animal after decapitation. The data obtained were analyzed by Student's *t* test. Samples for electron microscopy were immersed immediately in 2.5% cold glutaraldehyde in 0.1 *M* phosphate buffer solution and fixed for 2 hours. They were transferred to buffer solution for washing and postosmicated by 2% osmium tetroxide in the same buffer. After dehydration in ethanol, some of them were critical point dried, coated with gold, and then observed under an S-405 scanning electron microscope (Hitachi). The remaining samples were embedded in epoxy resin after dehydration. Ultrathin sections were made with an ultramicrotome and examined under an H-300 transmission electron microscope (Hitachi).

RESULTS

Control Group. On gross examination, no effusions were observed in the tympanic cavities of the

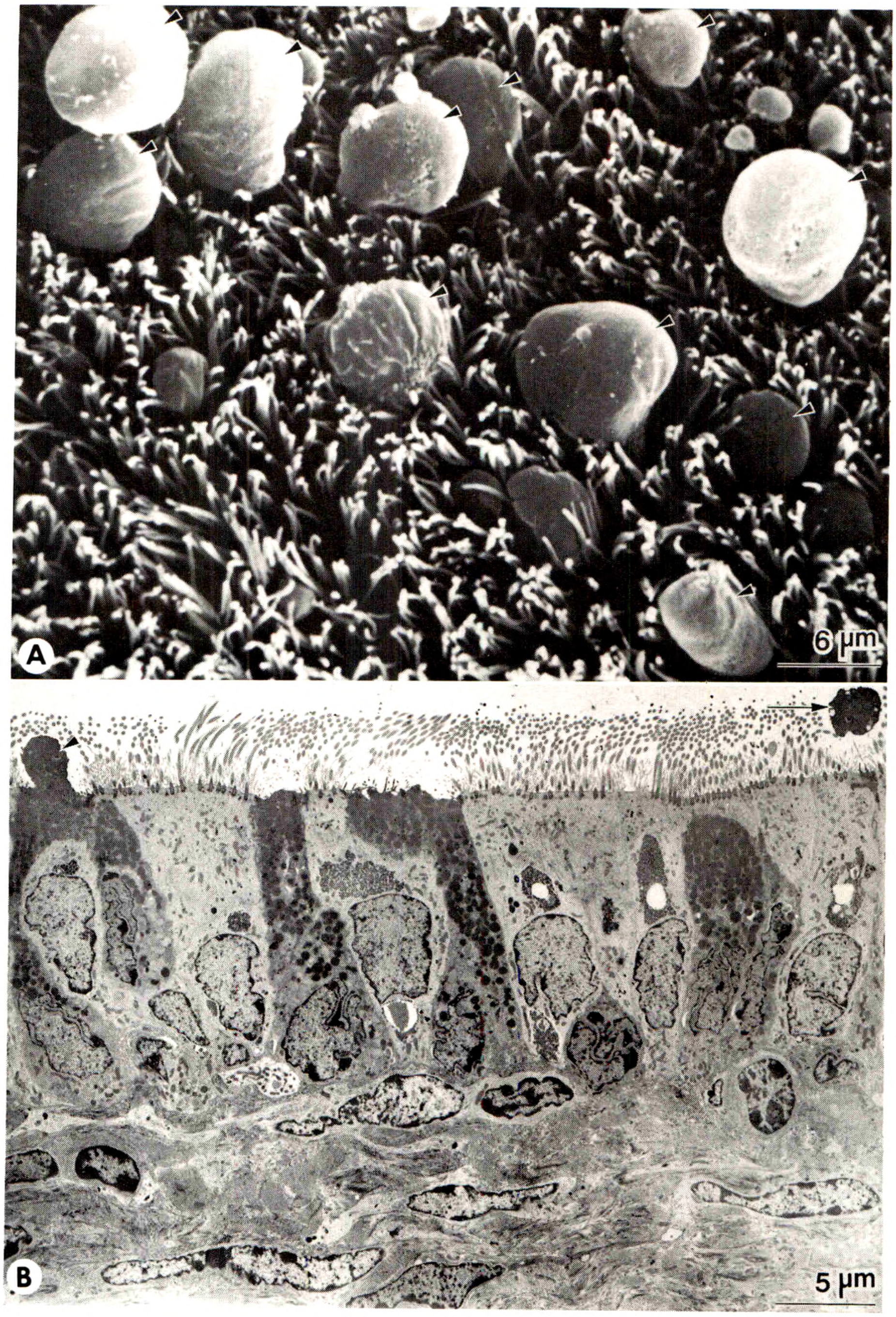

Fig 2. Proximal site of middle ear mucosa of animal exposed to sulfur dioxide. A) Scanning electron photomicrograph (original x1,500). Frequent ballooning of goblet cells (arrowheads) and sporadic secretory granules or droplets on mucosa are observed, but no disordered cilia. B) Transmission electron photomicrograph (original x1,000). Protuberances of goblet cells (arrowhead) and sporadic apocrine secretions (arrow) are seen.

control animals. The middle ear mucosae seemed normal. The ciliary activities (mean ± SD) of ten animals were 747 ± 28 beats/min in the proximal site and 945 ± 31 beats/min in the distal site. Electron microscopy showed few abnormal findings in the middle ear mucosa (Fig 1). The ciliated cells showing pathologic signs such as compound cilia and cytoplasmic vacuolation were less than 1% in the proximal site and less than 2% in the distal site.

Experimental Group. On gross examination, no effusions were found in the tympanic cavities of ten guinea pigs, but the middle ear mucosae were edematously swollen. The ciliary activities (mean ± SD) of this group were 724 ± 31 beats/min in the proximal site and 498 ± 33 beats/min in the distal site. The ciliary activity in the proximal site of this group was not different from that of the control group. On the other hand, the ciliary activity in the distal site was significantly less than that of the control group ($p < .0005$).

Morphologically, the most conspicuous finding in the proximal site was hypersecretion: ballooning of goblet cells (Fig 2), discharge of secretory droplets (Fig 2A), and frequent apocrine secretions were observed. Pathologic cilia such as compound cilia or short cilia were rarely observed in the proximal site. However, vacuolation of epithelial cells and expansion of intercellular spaces were found sporadically

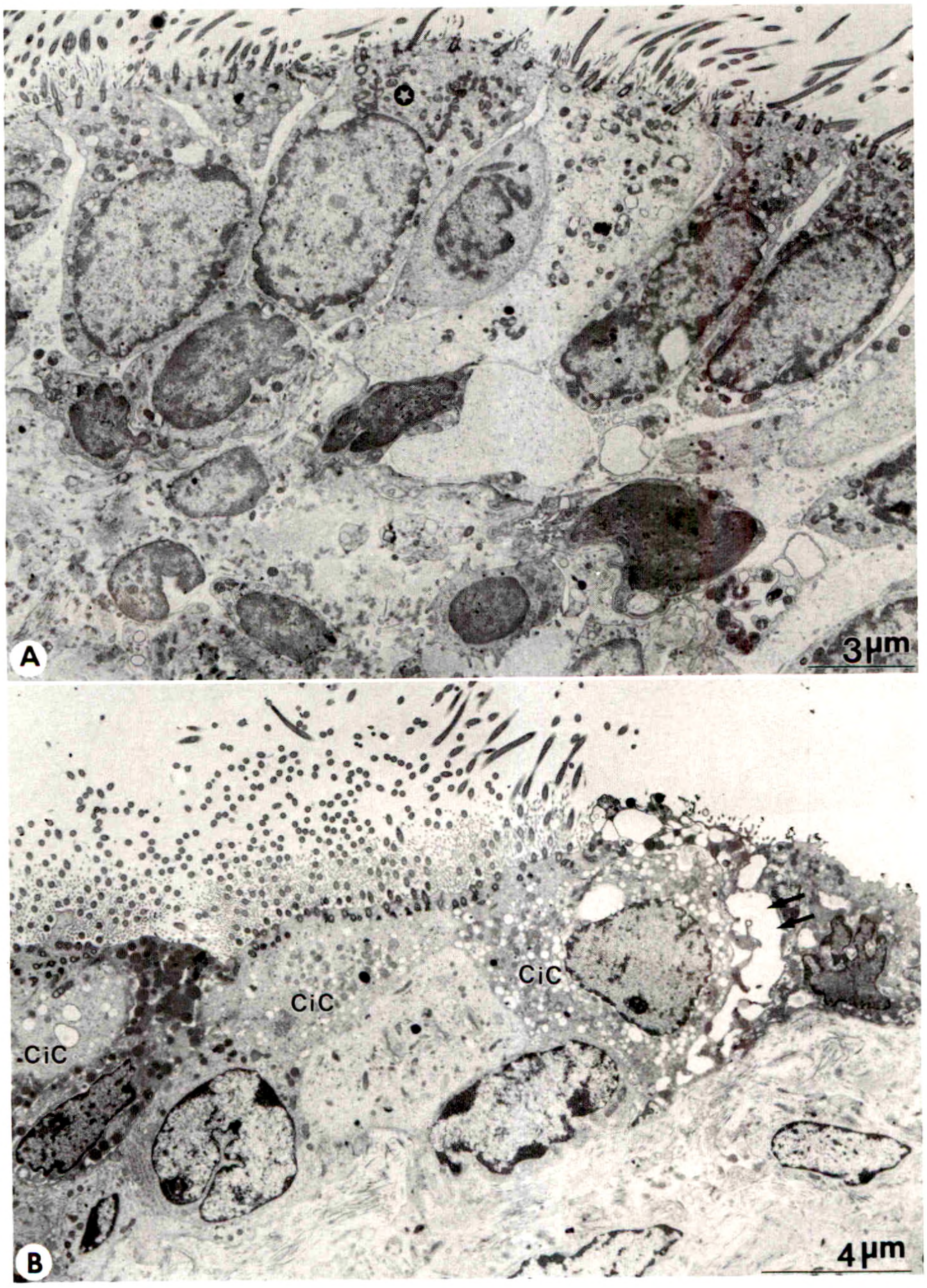

Fig 3. Transmission electron photomicrographs of middle ear mucosa of animal exposed to sulfur dioxide. A) Proximal site (original x2,000). Abnormal cilia such as compound cilia are not observed, but ciliated cells with decreased number of cilia (star) and vacuolation of ciliated cells are shown. Sporadic expansion of intercellular spaces is also observed. B) Distal site (original x3,000). Frequent vacuolation of ciliated cells (CiC) and sporadic expansion of intercellular spaces (arrows) are seen.

in the proximal site (Fig 3A). Approximately 4% of ciliated cells in the proximal site showed pathologic changes.

On the other hand, electron microscopy showed rather severe pathologic changes in the distal site. These changes include frequent vacuolation of ciliated cells (Fig 3B), expansion of intercellular spaces (Fig 3B), and cytoplasmic protuberances. In addition, loss of cilia, formation of compound cilia (Fig 4A), and falloff of epithelial cells (Fig 4B) were sporadically observed. Over 25% of ciliated cells in the distal site showed pathologic changes.

DISCUSSION

Mucociliary function is a critical primary defense mechanism of the respiratory tract, as well as the middle ear, in both animals and humans. Along with other host defense mechanisms, mucociliary transport function plays a role in keeping the middle ear or the lower airway sterile. It has been suggested on the basis of morphologic studies that disruption of the mucociliary function could lead to the development of middle ear effusions.[18-20] However, our knowledge of the mucociliary function in the tubotympanum is still limited. It has been only relatively recently that the mucociliary system has been considered to play an important role in the defense system of the middle ear. Sadé[21] demonstrated, by tracing the movement of foreign materials, that the middle ear mucosa has an active transport function. Hadas and Sadé[22] measured the mucociliary clearance rate in patients with middle ear

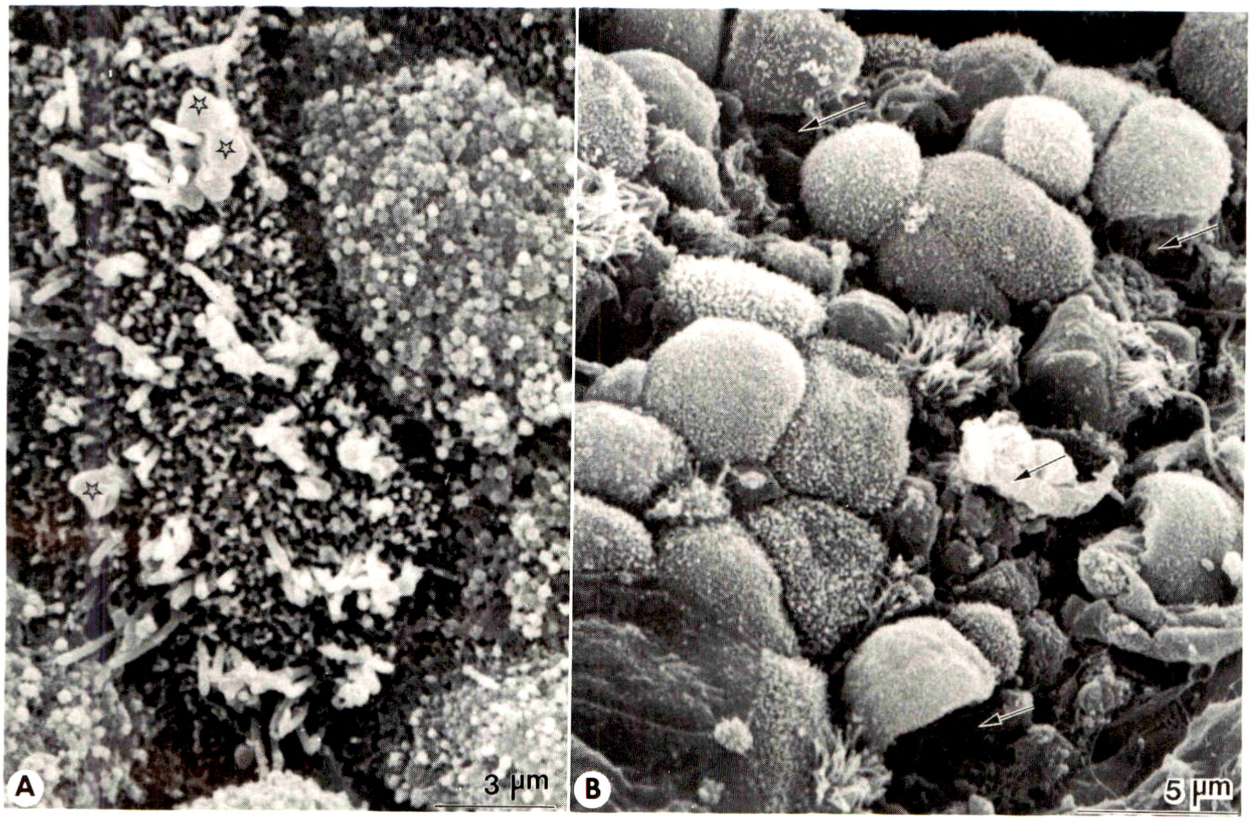

Fig 4. Scanning electron photomicrographs of distal site of middle ear mucosa of animal exposed to sulfur dioxide. A) In ciliated cells, decreased number of cilia is observed, as well as sporadic formation of compound cilia (stars) (original x5,000). B) Surface induration of mucosa and sporadic falloff of epithelial cells (arrows) are seen (original x1,500).

disease by the use of nonsoluble saccharin and showed that most ears with simple chronic otitis media or atelectasis have a patent eustachian tube through which the mucociliary system can transport foreign particles. They also documented that transport tended to be slowed if the ear was infected. Recent studies using a radioisotopic method have confirmed that the mucociliary function declines or is totally absent in the presence of otitis media with effusion (OME) and that the mucociliary transport returns to normal when the ear is clinically healed.[23,24] These studies have strongly suggested that the impairment of the mucociliary function in the tubotympanum plays an important role in the development and pathogenesis of middle ear diseases, especially OME.

It is well established that the mucociliary system in the respiratory tract is rather susceptible to noxious inhalants or atmospheric pollutants and that the pathologic changes observed in respiratory cilia in the chronic bronchitis of experimental animals exposed to these pollutants are similar to those seen in the middle ear of humans and experimental animals with OME.[25,26] Thus it may be that atmospheric pollutants affect the mucociliary system in the middle ear, resulting in middle ear effusions.

From a clinical viewpoint, it seems probable that OME is the result of a variety of causes. It is important to clarify the mechanisms responsible for the development and chronicity of this disease. Many epidemiologic studies have been undertaken to determine the predisposing factors of this condition on the basis of extensive clinical data. The environmental factors are the best documented: climate, contagion, pollution, hygiene, feeding, and standard of living. Pukander et al[27] found a higher incidence of OME in urban areas than in rural areas. A study by Harvery[28] in urban and rural parts of Ulster revealed that 13.1 per 1,000 children in the rural areas and 19.8 per 1,000 children in the urban areas had OME, and the areas of the greatest density of population and the highest degree of air pollution had the highest prevalence. A prospective study of 337 children was carried out by Iversen et al[29] to evaluate the importance of indoor environmental factors in homes for the incidence of middle ear effusions. They found that parental smoking at home increased the frequency of middle ear effusions in children. These reports might suggest a probable role of atmospheric pollution in the pathogenesis of OME. However, there is disagreement on this point.[30,31] In addition, even if atmospheric pollution does contribute to OME, there is the possibility that it might be mediated by events entirely outside the middle ear: respiratory mucosa damaged by such pollution might be more susceptible to upper respiratory tract infections, which in turn are associated with OME.

Very few experimental studies are available on the effect of atmospheric pollution on the middle ear. In our present study, we chose to study the acute effects of sulfur dioxide exposure because it is one of the most representative atmospheric pollutants. We previously studied the influence of 24 hours of exposure to 300 ppm of sulfur dioxide on the upper and lower respiratory tract (unpublished observations). For purposes of comparison, the same concentration and exposure period were used in this study.

In our present study, the ciliary activities immediately after the exposure period were 724±31 beats/min in the proximal site and 498±33 beats/min in the distal site. On the other hand, the ciliary activities after the exposure to gas containing air alone were 747±28 beats/min in the proximal site and 945±31 beats/min in the distal site. According to the present control data and our previous control data collected from healthy guinea pigs,[14,32] the results obtained this time show that the ciliary activity is not reduced in the proximal site but apparently is impaired in the distal site: marked discrepancy in the susceptibility of the two locations was observed. We have already reported similar discrepancies in reactions to irradiation,[33,34] infectious pathogens,[15,35,36] and toxic chemicals.[37] Therefore, it is logical to assume that cilia in the distal site are much more fragile than those in the proximal site. The present electron microscopic examinations demonstrated that cilia or ciliated cells present in the distal site were damaged much more severely than those in the proximal site, which corresponds well to our functional data.

The most dramatic morphologic change observed was hypersecretion in the proximal site, which might be consistent with a protective function. Both ciliostasis[5,6] and stimulation of mucus-secreting structures[38] in the lower respiratory tract have been demonstrated under experimental conditions by use of high-level exposure to sulfur dioxide. The hypersecretion observed in the middle ear is consistent with the pathologic changes reported in the lower respiratory tract. We observed sloughing of epithelial cells and loss of ciliary activity in the nasal mucosa immediately after 24 hours of exposure to 300 ppm of sulfur dioxide (unpublished data), but the pathologic changes occurring in the middle ear were much milder. We found no middle ear effusions in our models, despite hypersecretion. This might mean that normally functioning cilia in the proximal site can prevent a retention of surplus secretions in the ear, even if the ciliary function in the distal site is inadequate.

It is generally agreed on the basis of both clinical[39,40] and experimental[41,42] data that bacteria or bacterial components (endotoxin or lipopolysaccharide) are involved in the pathogenesis of OME. Functional and structural abnormalities resulting from short-term exposure to high concentrations of sulfur dioxide impair pulmonary defense mechanisms against bacterial and viral infections by decreasing the clearance of streptococci from the lung[43] and favoring the development of pneumonia after exposure to virus.[44] Our present study demonstrated, in the middle ear distal to the tympanic orifice, functional and structural abnormalities that might also contribute to bacterial infection and lead to middle ear effusions. In addition, there is another way for sulfur dioxide exposure to contribute to middle ear effusions. If bacterial infections were present in the middle ear before exposure, short-term exposure to high concentrations of sulfur dioxide could lead to middle ear effusions, because bacteria and lipopolysaccharide reduce the ciliary activity[15,35,36] and sulfur dioxide stimulates the mucus-secreting structures in the middle ear.

Short-term exposure to 300 ppm of sulfur dioxide did not develop middle ear effusions, but hypersecretion and reduced ciliary activity in the middle ear distal to the tympanic orifice were observed. The possibility cannot be ruled out that OME might be induced after exposure to sulfur dioxide, because some kinds of environmental pollutants have delayed effects on the mucociliary system.[45,46] The concentration of 300 ppm used in this study is over ten times the concentration observed in major metropolitan areas with industrial as well as automobile pollution. Further experiments on the effect of sulfur dioxide at a lower concentration, or on the effect of longer-term exposure, will be necessary to establish the involvement of atmospheric pollution in the development and chronicity of OME.

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BRAIN STEM AUDITORY EVOKED POTENTIALS IN JAUNDICED GUNN RATS

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Bilirubin encephalopathy causes potentially preventable brain damage and hearing loss. The site of auditory dysfunction is controversial, despite pathologic studies showing damage to brain stem auditory nuclei in humans and experimental animals. We studied the effects of bilirubin toxicity on the auditory system of homozygous jaundiced Gunn rats by use of brain stem auditory evoked potentials. Small but statistically significant abnormalities were found for wave latencies, interwave intervals, and amplitudes.

KEY WORDS — bilirubin encephalopathy, brain stem auditory evoked potentials, hearing loss, hyperbilirubinemia, kernicterus.

INTRODUCTION

Bilirubin encephalopathy, kernicterus, and hearing loss are all significant and potentially preventable sequelae of hyperbilirubinemia suffered by premature and low birth weight infants treated in neonatal intensive care units.[1-6] Bilirubin toxicity in humans produces multiple neurologic deficits[7-10] and hearing loss.[2,4,11-14] The site of auditory dysfunction with bilirubin toxicity is controversial. Pathologic studies of jaundiced infants who died with kernicterus show consistent abnormalities of the auditory brain stem nuclei and fiber tracts, including the cochlear nuclei, trapezoid bodies, lateral lemnisci, superior olives, and inferior colliculi,[1,15-19] whereas available information limited to light microscopy suggests sparing of the auditory nerve[17] and inner ear structures[15,16,18] or, in one case only, low grade abnormalities of the inner ear.[20]

In contrast, audiologic measures are consistent with inner ear and/or CNS abnormalities. The evidence for a cochlear abnormality with bilirubin toxicity includes the findings of a predominantly high frequency loss, usually bilateral and symmetric, with recruitment and type II Bekesy tracings.[11,12,14,21,22] However, Carhart[23] and Dublin[15] interpret the audiometric findings to indicate a lesion in the cochlear nucleus. Central nervous system abnormalities, either alone or in combination with sensory loss, are suggested by reports of decreased binaural fusion, auditory aphasia and imperception, word deafness, and numerous instances of patients labeled as "deaf" when objective tests show normal thresholds.[13,21,24-27]

Brain stem auditory evoked potentials (BAEPs) have contributed physiologic information both in patients with chronic bilirubin encephalopathy and in neonates with acute hyperbilirubinemia. Studies of children and adults with hearing loss following hyperbilirubinemia show both peripheral (cochlea and/or auditory nerve) and central (brain stem) auditory dysfunction based on 1) increased latency of wave I,[28] 2) latency and amplitude abnormalities of waves II,[29] III,[30] and V,[29,31] and 3) interwave interval abnormalities of I-III,[30,31] I-(−V), and III-(−V) (negative waves are troughs).[31] Nakamura et al[28] have found a positive correlation between BAEP latency abnormalities and measurements of total and unbound bilirubin. Reversibility of BAEP abnormalities following exchange transfusion for neonatal jaundice has also been described.[28,31-34]

A major problem in understanding the pathogenesis of bilirubin encephalopathy is separating the effects of bilirubin damage from other potentially harmful factors (eg, hypoxia, ischemia, acidosis, aminoglycoside exposure) prevalent in newborn intensive care units. One value of animal models is that these variables can be eliminated or controlled. To study the effects of bilirubin toxicity on the auditory system, we used BAEPs in jaundiced Gunn rats.

The Gunn rat model of unconjugated hyperbilirubinemia is a widely accepted model for human bilirubin encephalopathy. The Gunn rat strain carries a single autosomal recessive genetic defect.[35] The Gunn rat's deficient enzyme, glucuronyl transferase, is the same enzyme as that responsible for hyperbilirubinemia in human neonates. Rats homozygous for the mutant gene (jj) have virtually no hepatic glucuronyl transferase activity[36] and are unable to conjugate free bilirubin. The jj rat is invariably jaundiced, is often ataxic, and is at risk for developing fatal bilirubin encephalopathy (kernicterus) in the first few months of life when the serum total indirect bilirubin concentration is maximal.[37,38] Adult survivors usually have residual ataxia and mild jaundice[37] (serum bilirubin concentra-

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TABLE 1. LITTER COMPOSITION OF STUDY GROUPS

	Litter								
Genotype	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Mean Weight (g)*	*Mean Age (d)*
JJ	—	—	—	—	—	MMM	M	474	241
	—	—	—	—	—	FFF	F	333	
Jj	M	M	M	—	—	—	—	423	138
	F	F	F	F	F	—	—	239	
jj	M	M	M	M	—	—	—	401	138
	F	F	F	F	—	—	—	219	
Age (d)	139	139	137	136	141	246	226	—	172

There are seven matched pairs of jj and Jj rats. Six of eight JJ rats are from single litter. Note that JJ rats are significantly older and heavier than others. JJ — normal, unaffected homozygotes, Jj — heterozygotes, jj — rats homozygous for mutant gene, M — male, F — female.

tion about 6 mg%). Thus, these rats have both acute and chronic bilirubin exposure. Heterozygotes (Jj) have approximately 50% glucuronyl transferase activity,[36,39] normal (< 1.0 mg%) serum bilirubin concentration,[37,38] and no phenotypic abnormalities. Normal, unaffected homozygotes (JJ) have normal (100%) enzyme activity and no abnormalities.

Pathologic studies in jaundiced Gunn rats demonstrate bilirubin staining throughout the brain, with areas of cell loss and gliosis in the brain stem, cerebellum, and basal ganglia.[38,40,41] The auditory nervous system is involved prominently, especially the cochlear nuclei.[40-43] The inner ear structures do not appear to be affected,[44,45] although detailed studies using modern histologic techniques are lacking. Neuropathologic changes with age[37,41,42,46,47] suggest that susceptibility to bilirubin toxicity is greater in the immature CNS.

Brain stem auditory evoked potentials are well suited to the study of bilirubin toxicity, since they can approximate anatomic localization within the auditory system and they permit noninvasive serial studies. Studies of BAEPs in Gunn rats have found both normal[48] and abnormal auditory function.[45] To help resolve this conflict, and as a prelude to more invasive studies in which bilirubin toxicity was increased by the administration of exogenous agents (eg, sulfonamides), we studied auditory responsiveness in the untreated Gunn rat as a function of genotype.

MATERIALS AND METHODS

A Gunn rat colony was established with breeders obtained from Dr Gerard Odell (University Hospital, University of Wisconsin, Madison). Breeding jaundiced jj males with Jj females yielded mixed litters of Jj and jj offspring. The jaundiced jj homozygous offspring were identified in the first few days of life by their yellowish color and marked by ear punching to distinguish them from their nonjaundiced Jj heterozygote littermates. The jj animals were selected from five litters, each with at least one jj male, one jj female, one Jj male, and one Jj female. Litters containing all three genotypes, obtainable from breeding Jj and Jj parents, are not bred in our colony because Jj rats can be distinguished from JJ rats only with great difficulty. Therefore, the four female and four male adult JJ rats were selected from two litters separate from those from which the jj and Jj rats were obtained. The data from eight of 32 rats were excluded because of elevated BAEP thresholds (see below). Of the remaining 24, the JJ rats (241 ± 11.8 days) were older than the Jj and jj rats (138 ± 1.6 days) ($p < .01$; Table 1). The body weights of jj and Jj rats were not significantly different, but the older JJ rats were heavier than both Jj and jj animals, and males were heavier than females ($p < .01$, Student's two-tailed t test).

Animals were anesthetized with intraperitoneal or intramuscular ketamine (40 mg/kg) and acepromazine (4 mg/kg). A rectal temperature probe was inserted, and the body temperature was kept constant at 39.5°C to 40°C with the use of a temperature controller (YSI model 73A, Yellow Springs Instrument Co, Yellow Springs, Ohio), heating blanket, and lamp. This temperature range was chosen after measurements made on eight normal, unanesthetized, restrained, adult rats yielded a mean of 39.9°C ± 0.28°C.

Subcutaneous platinum electrodes (Grass type E2) were inserted 1) at the vertex (where an imaginary line between the posterior canthi of the eyes crossed the midline) and advanced 1 cm forward subcutaneously, and 2) at the right and left mastoids 0.3 cm below the external ear canal and advanced anterolaterally. A ground electrode was placed in the tail or leg. Impedance was at or below 3,000 Ω.

Sound stimuli were delivered monaurally through a Sony Walkman 4LIS earphone speaker chosen because of its small size and good frequency response (Fig 1). The speaker was centered over and just touching the entrance to the right external ear canal. Care was taken to insure that the ear canal was not collapsed.

Two simultaneous differential recordings were obtained: one from the vertex to the right mastoid (CZ-A2), and one from the left to the right mastoid (A1-A2). Right (ipsilateral) mastoid negativity was displayed upward. The signal was amplified (10^5)

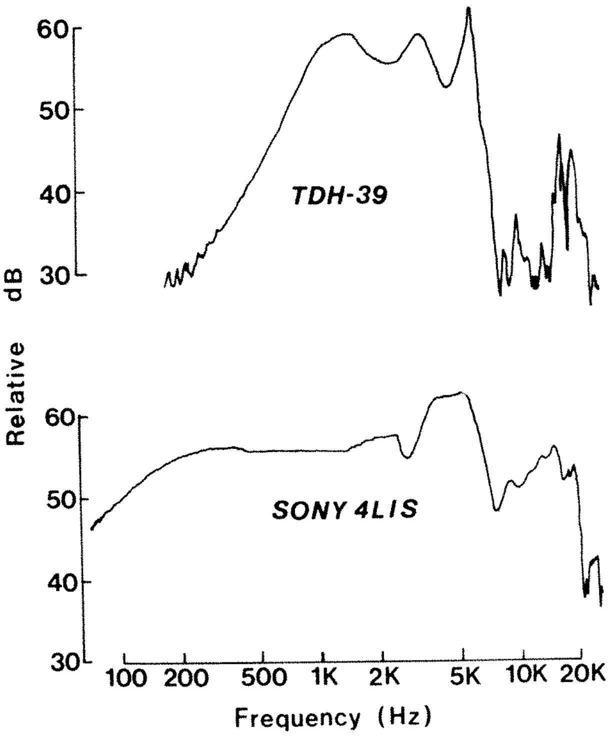

Fig 1. Comparison of Sony MDR-4LIS and TDH-39 earphones using real ear calibration in adult human volunteer. Bruel and Kjaer Heterodyne analyzer swept through 20 to 22,000 Hz with 100-mV sine wave inputted into speakers; and B & K ¼-in microphone at entrance to ear canal measured sound pressure, which was graphed on B & K graphic level recorder.

and filtered from 30 to 3,000 Hz (6 dB/octave rolloff). Signals were averaged on a Nicolet 1170 evoked potential averaging system (Nicolet Instruments, Madison, Wis) and stored on magnetic disc. Each waveform consisted of the averaged response to at least 1,024 stimuli, and at least two replications were performed for every condition.

All recording was done in a sound attenuated booth (model AC-3, Industrial Acoustic Company, New York). The stimuli were 100-μs clicks. Sound intensity was calibrated in the sound attenuated booth by placing the speaker 0.5 cm (to approximate the length of the rat ear canal) from a ½-inch B & K (Bruel and Kjaer, Naerum, Denmark) type 4155 prepolarized condenser microphone attached to a B & K precision integrating sound level meter, type 2233, set at impulse weighting, peak detecting, linear scale (equal to peak equivalent sound pressure level [SPL] minus 3 dB,[49] ie, 75 dB impulse-weighted peak-detection SPL = 72 dB peak equivalent SPL). Using the same signal conditions, the psychoacoustic click threshold for three human adults with normal audiograms was 30 dB SPL.

Three stimulus conditions were presented in random order. Click intensities of 75 and 45 dB SPL, 50 and 20 dB above the electrophysiologic threshold of a group of normal adult rats, were presented at a stimulation rate of 33.5/s. A condition of 75 dB SPL

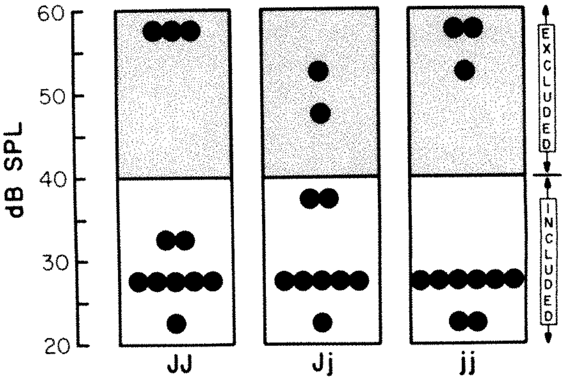

Fig 2. Thresholds for 32 Gunn rats in dB SPL (for calibration, see text). Cutoff of 40 dB SPL was used for inclusion in study. Distribution of excluded rats (three JJ, two Jj, and three jj) does not indicate any genotype differences in BAEP threshold. In fact, included jj rats had thresholds as good as or better than other groups. Threshold was bracketed within 5-dB steps.

at a rate of 88.5/s was also included. To insure response stability throughout the testing, these conditions were preceded and followed by additional recordings at 75 dB SPL at 88.5/s, and data were accepted only if the pretest to posttest changes in latency of waves I, II, and III were less than 0.04 ms.

Each rat's BAEP click threshold was estimated to within 5 dB by lowering intensity in 10-dB steps from 75 dB SPL at 88.5/s. When the entire BAEP response disappeared, intensity was then raised and lowered in 5-dB steps and the number of stimuli obtained for each replication was doubled or quadrupled. The electrophysiologic threshold was thus bracketed within 5-dB steps.

An electrophysiologic threshold of less than 40 dB SPL was chosen as a criterion for inclusion in the study. Of 32 rats tested, 24 met this criterion, eight in each genotype. The excluded rats, with an increase of about 30 dB in their electrophysiologic thresholds (Fig 2) were believed to have middle ear dysfunction. In separate observations of 30 Gunn rat ears, there was a 20% prevalence of middle ear disease evenly distributed across genotype as evidenced by the concurrence of an elevated BAEP threshold, abnormal mobility of the tympanic membrane by pneumatic otoscopy, and thick, viscous fluid found in the middle ear on dissection of the bulla.

The dependent variables were analyzed with a two-way analysis of variance and covariance (BMDP2V, BMDP Statistical Software, Berkeley, Calif, 1983). Genotype and sex were the group variables, and weight was the covariable. Welch's approximation for unequal variance was used for post hoc two-sample, two-tailed t tests (Minitab, Inc, University Park, Pa, 1985).

Our choice of sample size of eight per group was based on the number needed to detect a 0.1-ms la-

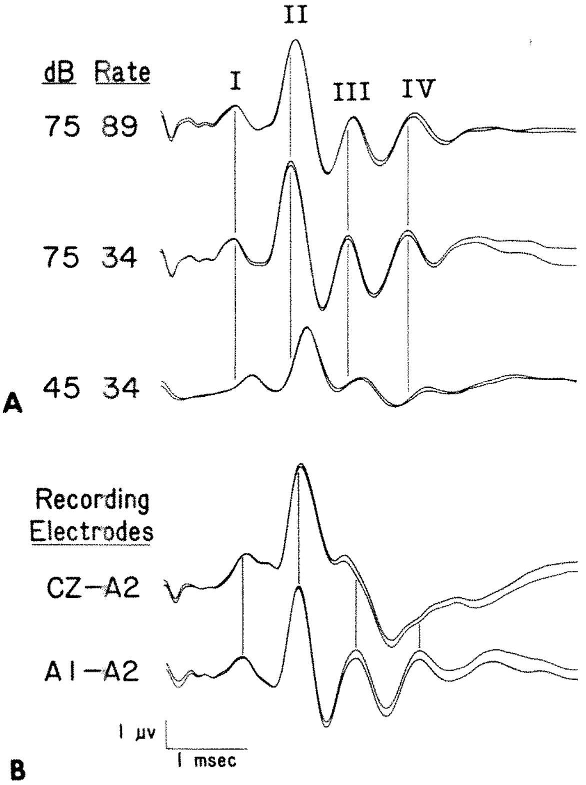

Fig 3. BAEPs to right ear stimulation. Two separate BAEP responses to 1,024 stimuli are superimposed. A) Differences in latency and amplitude seen at two different stimulus intensities (75 and 45 dB SPL) and different rates (33.5/s and 88.5/s) in A1-A2 electrode recording montage. Note latency delays of later waves to 75-dB, 88.5/s stimulation, and latency delays of all waves in 45-dB, 33.5/s condition. Vertical lines indicate latencies of waves in 75-dB, 33.5/s stimulus condition. These BAEPs are from one JJ rat. B) Simultaneously recorded responses from CZ-A2 and A1-A2 in one Jj rat for same stimulus conditions (75 dB and 33.5/s). Note better resolution of waves in A1-A2 recording montage.

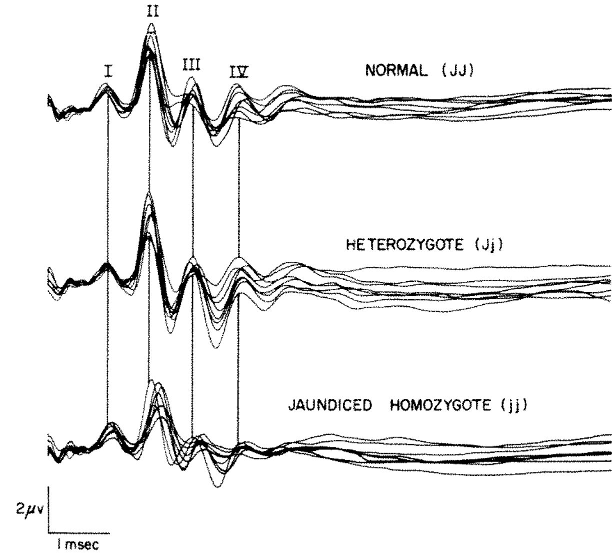

Fig 4. Individual BAEPs from 24 Gunn rats segregated by genotype. Each genotype grouping consists of eight BAEPs of 2,048 sweeps each, all at 75 dB SPL, 33.5/s in A1-A2 montage only. Vertical lines indicate means for waves I, II, III, and IV in heterozygotes. Note variability in jj group, especially in wave II. Responses with lower amplitude have delayed latencies; this relationship not present in other two groups. Mean latencies of I, II, and III are prolonged in jj group compared to JJ and Jj groups.

tency difference between two groups with an estimated standard deviation of 0.1 ms, an α level of 0.05, and a power of 0.8.[50] With a standard deviation of 0.07, which was greater than that which we found for most latency and interwave interval-dependent variables, we should have a power of 0.95 to detect a 0.1-ms difference between two groups. Of course, this means that we expect to miss 5% of real differences, and that 5% of our identifications will be due to chance alone.

RESULTS

Representative BAEPs to right ear stimulation of one normal JJ rat in the A1-A2 electrode recording montage (Fig 3A) illustrate the latency and amplitude changes seen at different intensities and rates of stimulation. Waves that are difficult to identify in one montage are often more prominent in the other. The differences between A1-A2 and CZ-A2 for a Jj rat (Fig 3B) illustrate the differences in BAEP appearance between the two montages. Overall, the A1-A2 montage gives more prominent and reliable peaks. The CZ-A2 montage is used to resolve the assignment of wave numbers in difficult or pathologic cases, and it helps to detect the presence of a BAEP response at near-threshold intensities since wave II in CZ-A2 is often the last wave to disappear.

Eight individual BAEPs for each of the three genotype groups are superimposed (Fig 4) to show the latency and amplitude differences between groups, and an interaction between latency and amplitude for waves I and II in the jj group, ie, the smaller amplitude responses in the jj but not the JJ and Jj groups are associated with an increase in latency. The spectrum of abnormalities in jj rats (Fig 5) ranges from normal (Fig 5B) to increasingly abnormal BAEPs (Fig 5C,D).

Genotypic Differences. The two-way ANOVAs with covariance showed a significant ($p < .01$) effect of genotype at 75 dB SPL, 33.5/s for the following dependent variables: 1) latency of waves I, II, and III in the A1-A2 montage, and II in CZ-A2; 2) interwave intervals I-II and II-IV in A1-A2, and I-II and II-III in CZ-A2; 3) amplitude of waves II and IV in A1-A2, and I in CZ-A2; and 4) amplitude ratios of II/I and IV/I in A1-A2, and II/I and III/I in CZ-A2 (Table 2).

The most consistent findings were that wave II latencies and I-II interwave intervals increased, and that wave II/I amplitudes decreased in jj rats in both electrode montages (Table 2). This indicates central (brain stem) auditory dysfunction, probably at the level of the cochlear nucleus.

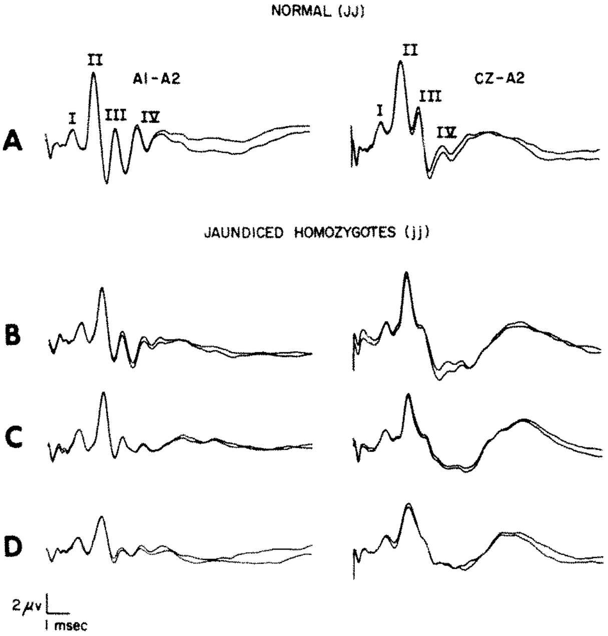

Fig 5. Spectrum of abnormalities in jaundiced homozygote (jj) is illustrated in three rats, all recorded with same stimulus conditions (75 dB SPL, rate 33.5/s, 1,024 sweeps and two replications for each animal). A) Normal control recording. Left panel is response from A1-A2 electrode pairing, two replications of 1,024 clicks each; right panel is recorded simultaneously from CZ-A2 electrode montage. B-D) Recordings obtained under same conditions as above from three different jj rats arranged in order of increasing abnormality. Note decreasing amplitude of waves II, III, and IV, seen especially well in A1-A2 recordings, and virtual disappearance of III and IV in CZ-A2. Note also broadening of base of peak II in both A1-A2 and CZ-A2 recordings — an observation we have noted but not quantified. See text for further discussion of these changes.

The A1-A2 montage may be more sensitive to genotype differences than the CZ-A2. Wave I and III latencies increased, and wave IV and IV/I amplitudes decreased in jj rats only in the A1-A2 montage (Table 2).

Two unusual amplitude findings are related: the significant effects of genotype on wave I amplitude and II-I amplitude ratio in CZ-A2 are both due to a decrease of wave I amplitude in the Jj group. The reason for this difference is not clear.

The percentage of total variance due to genotype ranged from 40% to 71% for the significant dependent variables. There were no significant effects for sex, weight, or the interaction between genotype and sex (with two exceptions noted below) in any of the latencies or amplitudes in either montage.

Wave I. The latency of wave I showed group differences in both the horizontal and vertical montage, accounting for 55% and 37% of the variance, respectively. Post hoc analysis showed that the jj latencies were prolonged compared to JJ ($p < .0001$), and Jj ($p < .0001$ for A1-A2 and $p = .014$ for CZ-A2). There was also a difference between Jj and JJ in the CZ-A2 montage ($p < .0001$). The topographic dependence of findings will be discussed later.

There were no significant group differences in wave I amplitude in A1-A2. A significant effect of genotype on wave I amplitude ($p = .003$) in CZ-A2 only is due to a very small amplitude in the Jj group only, compared to JJ and jj ($p = .002$ and $p = .02$, respectively). An explanation for this finding is not apparent.

Wave II. There was a significant effect of genotype on the latency of wave II in both A1-A2 and CZ-A2 montages (Table 2). Genotype accounted for 66.3% and 72.9% of the variance, respectively. Post hoc analysis revealed prolonged wave II for the jj group compared to both the JJ and Jj groups in both montages ($p < .0001$ for all comparisons).

The I-II interwave interval was significantly different in both montages (Table 2), with genotype accounting for 44% and 66.9% of the variance in A1-A2 and CZ-A2, respectively. These differences were due to prolonged I-II interwave intervals in the jj group, a 10.4% and 16.6% increase over the Jj group in A1-A2 and CZ-A2, respectively.

There was an effect of genotype on the amplitude of wave II in A1-A2 ($p = .003$, Table 2) and CZ-A2 ($p = .007$) that accounted for 42.3% and 66.1% of the variance, respectively. Post hoc comparisons showed a smaller wave II for the jj group. Analysis of the ratio of II/I was used to reduce the between-subject variability of absolute amplitude. This increased the significance of p values (Table 2) and the percentage of variance due to genotype to 49.4% in A1-A2 and 72.5% in CZ-A2. Post hoc analysis showed a diminished II/I ratio for the jj group compared to the Jj group in both montages ($p < .0001$).

There was also a significant genotype-by-sex interaction for II/I in the vertical montage ($p = .0008$; 14.8% of variance). The smaller Jj wave I amplitude in CZ-A2 noted previously occurred in the males of the Jj group and accounted for an increased II/I ratio in Jj males.

Wave III. Wave III latency showed a significant effect of genotype accounting for 68.3% of variance only in the A1-A2 montage. This was due to prolonged latency in the jj group.

The II-III interwave interval showed a significant effect of genotype in CZ-A2 (44.5% of variance). This was due to a shortened rather than prolonged II-III interval for the jj compared only to the JJ group. Neither the I-III nor the I-IV interwave interval was increased, despite the increased I-II. This suggests that waves III and IV in the rat may not be generated in series with waves I and II, ie, the neurons that generate waves III and IV may lie in pathways parallel to those that generate wave II and/or wave I. However, it is also possible that the

TABLE 2. LATENCY, INTERWAVE INTERVALS, AMPLITUDE, AND AMPLITUDE RATIOS WITH GENOTYPE AND ELECTRODE MONTAGE

Wave Latencies (ms)		*Wave I*		*Wave II*		*Wave III*		*Wave IV*					
Montage	*Genotype*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*				
A1-A2	JJ	0.95	.03	1.64	.03	2.31	.04	3.06	.06				
A1-A2	Jj	0.96	.02	1.64	.02	2.30	.03	3.10	.05				
A1-A2	jj	1.04	.03	1.78	.06	2.44	.06	3.14	.05				
		p = .0006		p< .0001		p = .0004		NS					
CZ-A2	JJ	1.00	.02	1.63	.03	2.28	.04	3.07	.06				
CZ-A2	Jj	1.04	.01	1.67	.03	2.26	.02	3.27	.15				
CZ-A2	jj	1.08	.03	1.80	.04	2.32	.06	3.42	.23				
		NS		p< .0001		NS		NS					
Interwave Intervals (ms)		*I-II*		*II-III*		*III-IV*		*II-IV*		*I-III*		*I-IV*	
Montage	*Genotype*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
A1-A2	JJ	0.69	.02	0.67	.04	0.76	.03	1.43	.05	1.36	.05	2.12	.06
A1-A2	Jj	0.67	.02	0.70	.02	0.76	.05	1.47	.04	1.38	.04	2.14	.05
A1-A2	jj	0.74	.03	0.66	.03	0.69	.06	1.36	.08	1.41	.04	2.10	.06
		p = .003		NS		NS		p = .005		NS		NS	
CZ-A2	JJ	0.66	.03	0.62	.02	0.78	.07	1.41	.06	1.28	.04	2.07	.07
CZ-A2	Jj	0.62	.03	0.59	.04	1.01	.16	1.30	.14	1.22	.02	2.22	.15
CZ-A2	jj	0.72	.04	0.52	.04	1.10	.25	1.62	.23	1.24	.05	2.34	.22
		p = .0001		p = .004		NS		NS		NS		NS	
Wave Amplitudes (μV)		*Wave I*		*Wave II*		*Wave III*		*Wave IV*					
Montage	*Genotype*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*				
A1-A2	JJ	1.82	.29	7.01	1.60	3.45	0.91	1.29	.55				
A1-A2	Jj	1.71	.56	8.29	1.70	4.03	1.98	1.46	.20				
A1-A2	jj	1.93	.62	5.33	1.69	2.02	1.19	0.61	.23				
		NS		p = .003		NS		p = .002					
CZ-A2	JJ	1.31	.35	5.60	1.88	4.86	1.47	0.82	.44				
CZ-A2	Jj	0.71	.27	6.09	1.22	6.00	1.42	0.39	.37				
CZ-A2	jj	1.14	.38	4.00	1.58	3.79	1.69	0.45	.36				
		p = .003		NS		NS		NS					
Amplitude Ratios				*II/I*		*III/I*		*IV/I*					
Montage	*Genotype*			*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*				
A1-A2	JJ	—		3.94	1.14	1.95	0.68	0.71	0.37				
A1-A2	Jj	—		5.05	1.02	2.47	1.30	0.94	0.35				
A1-A2	jj	—		2.81	0.60	1.02	0.47	0.33	0.10				
				p = .001		NS		p = .003					
CZ-A2	JJ	—		4.34	1.61	3.88	1.34	0.72	0.52				
CZ-A2	Jj	—		9.50	2.64	9.51	3.37	0.75	0.92				
CZ-A2	jj	—		3.60	0.94	3.28	0.65	0.37	0.23				
				p< .0001		p< .0001		NS					

p — result of two-way ANOVA for main effect of genotype; NS — not significant, ie, $p \geq .01$; SD — standard deviation.

study sample size simply lacked the statistical power to detect actual I-III and I-IV differences.

While there were no significant wave III amplitude differences, the III/I amplitude ratio showed a significant difference in CZ-A2, with 71.3% of variance due to genotype, and another 16.9% due to a genotype-by-sex interaction (p = .0002). The III/I amplitude difference was due to the smaller wave I of the Jj males, and not to any difference in wave III amplitude (in contrast to the II/I amplitude difference, where a smaller jj wave II amplitude also contributed to the smaller II/I). Thus, several unusual amplitude findings are correlated; the significant effects of genotype on wave I amplitude and III/I amplitude ratio in CZ-A2, and the unusual genotype-by-sex interactions of II/I and III/I in this montage are all due to a decreased wave I amplitude in Jj males, the reason for which is not clear.

Wave IV. Wave IV latency showed no significant group differences. The III-IV and II-IV interwave intervals showed group differences due to genotype in A1-A2 (41.7% and 44.8% of variance, respectively). As with II-III, post hoc analysis showed that both the II-IV and III-IV interwave intervals were decreased rather than increased in the jj group.

Wave IV amplitude and the IV/I amplitude ratio showed a significant reduction in the jj group in A1-A2 only (47.5% and 43.6% of variance due to genotype, respectively). The IV/II and IV/III amplitude ratios showed no significant differences.

TABLE 3. LATENCY CHANGE OF FOUR WAVES AND TWO INTERWAVE INTERVALS IN A1-A2 ELECTRODE MONTAGE FOR 30-dB CHANGE OF INTENSITY (75 dB TO 45 dB SPL)

Genotype	*Wave*	*Mean**	*SD*†	±*99% CI*§
JJ	I	8.17	1.25	6.62, 9.71
	II	7.96	1.64	5.93, 9.98
	III	8.13	1.98	5.67, 10.58
	IV	10.29	1.50	8.43, 12.14
	I-II	− 0.21	0.64	−1.00, 0.58
	II-III	0.17	1.92	−2.21, 2.54
Jj	I	8.17	0.67	7.34, 9.00
	II	8.04	1.11	6.68, 9.41
	III	6.42	3.41	2.20, 10.64
	IV	10.83	1.82	8.58, 13.08
	I-II	− 0.13	0.69	−0.98, 0.73
	II-III	− 1.63	3.40	−5.84, 2.57
jj	I	8.21	2.13	8.21, 8.21
	II	8.08	1.72	5.96, 10.21
	III	7.25	4.19	2.06, 12.44
	IV	10.42	1.57	8.47, 12.36
	I-II	− 0.12	0.11	−0.26, 0.02
	II-III	− 0.83	4.67	−6.60, 4.94

Since only two intensities were studied, linearity of this function cannot be inferred.

*μs/dB. For each type and wave, n = 8.

†Standard deviation in μs/dB.

§99% confidence interval (two-tailed) about mean.

TABLE 4. LATENCY CHANGE OF FOUR WAVES AND TWO INTERWAVE INTERVALS IN A1-A2 FOR 55 SEC^{-1} CHANGE OF STIMULATION RATE (33.5 SEC^{-1} TO 88.5 SEC^{-1})

Genotype	*Wave*	*Mean**	*SD*†	±*99% CI*§
JJ	I	0.48	0.26	0.16, 0.79
	II	1.18	0.29	0.82, 1.54
	III	1.32	0.92	0.19, 2.45
	IV	1.52	0.60	0.78, 2.26
	I-II	0.70	0.21	0.45, 0.96
	II-III	0.14	0.96	−1.05, 1.33
Jj	I	0.39	0.15	0.20, 0.57
	II	0.89	0.42	0.37, 1.40
	III	1.27	0.55	0.59, 1.95
	IV	1.73	0.71	0.85, 2.60
	I-II	0.50	0.37	0.04, 0.96
	II-III	0.39	0.69	−0.47, 1.24
jj	I	0.52	0.23	0.24, 0.80
	II	1.14	0.29	0.78, 1.49
	III	1.39	0.38	0.92, 1.85
	IV	2.02	0.92	0.88, 3.17
	I-II	0.61	0.94	−0.55, 1.78
	II-III	0.25	0.32	−0.15, 0.65

Since only two rates were studied, linearity of this function cannot be inferred.

*μs/sec^{-1}. For each type and wave, n = 8.

†Standard deviation in μs/sec^{-1}.

§99% confidence interval (two-tailed) about mean.

Change in Latency With Intensity and Rate. The change of intensity from 75 dB SPL to 45 dB SPL caused an increased latency for all waves of about 240 μs, or 8 μs/dB (Table 3). There were no differences in latency shift with intensity between genotypes. The I-II and II-III interwave intervals were no different at these two intensities.

An increased rate of stimulation, from 33.5 to 88.5 clicks/s, produced increased latencies for all waves. The effects were greater on waves II, III, and IV than on wave I; therefore, the interwave intervals (eg, I-II) were also increased (Fig 6). There were no differences between genotypes in response to increased rate of stimulation (Table 4).

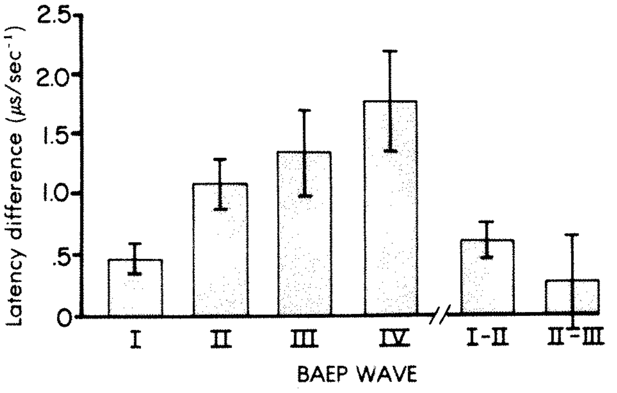

Fig 6. Latency change of waves I, II, III, and IV and interwave intervals I-II and II-III with rate of stimulation for all animals (n = 24). Difference in latency between two rates, 33.5/s and 88.5/s, is expressed in μs/sec^{-1} (see Table 4 for further detail). Means and 99% confidence intervals are shown. Note that latency changes are greater for waves II, III, and IV than for wave I, and that there is increased latency with increased rate of stimulation for all waves and for interwave interval I-II.

DISCUSSION

The homozygous Gunn rat is the best known model for human pathologic and biochemical changes due to bilirubin.[37,40,51] Despite the numerous anatomic and biochemical studies of bilirubin encephalopathy in the Gunn rat, few functional studies have been published. While behavioral studies[30,48] have shown normal auditory thresholds, the functional correlates of the known pathologic lesions of the auditory pathways are not well defined. This study examined Gunn rat genotype as an index of the effects of hyperbilirubinemia. The changes in the homozygous jj Gunn rats are assumed to be the result of this lifelong hyperbilirubinemia, although the bulk of the damage probably occurs early in life, when serum bilirubin is maximal. Damage due to inherited abnormalities of the auditory nervous system cannot be excluded, although we believe the weight of evidence favors bilirubin toxicity.

The changes found in jj Gunn rats are 1) wave I prolongation, 2) prolongation of the I-II interwave interval and decreased amplitude of II and II/I, and 3) interwave interval and amplitude ratio abnormalities of later waves.

The significant prolongation of wave I in the homozygotes suggests pathologic function of the cochlea, auditory nerve, or the synapse between the hair cell and the primary auditory neuron.[52] Conductive loss is not likely to be the cause of the prolonged wave I, since rats with elevated BAEP thresholds were excluded from the study. In agreement with previous studies,[45,48] there were no genotype differences in click thresholds in our rats. Though we doubt that animals with inner ear disease were excluded by our threshold criteria, we cannot deny this possibility. If this were true, then the amount of peripheral disease we identified underestimates the true amount.

The differences in the interwave intervals and amplitudes indicate central dysfunction. The increased I-II interwave interval and the decreased amplitude of II and II/I correlate with the known severe histologic abnormalities of the cochlear nuclei in the Gunn rat,[42,43] because wave II in the rat is generated primarily by synchronous neural activity in the cochlear nucleus.[52] The decreased amplitude of II replicates this finding of Uziel et al.[45]

We also found no increase in I-III and I-IV interwave intervals, despite a I-II increase in jj rats. This pattern of abnormality suggests that auditory dysfunction with bilirubin toxicity is more prominent early in the central afferent pathways, and that bilirubin toxicity selectively affects only a subset of parallel afferent pathways. Thus, a serial generator model of BAEP waves cannot be assumed.

Differences in morphology, amplitude, and variance as a function of montage may explain partly the topographic dependence of findings. All other things being equal, the experimental power to detect differences in any given BAEP wave may suffice in one projection and not another. Furthermore, there is no assurance that the same neuronal subpopulations are sampled with different montages. The design of this study does not resolve or directly address these issues.

Differences between JJ (normal) and Jj (heterozygote) rats have important implications from the standpoint of experimental design (ie, control group selection). Heterozygotes had a longer latency and lower amplitude of wave I, and a longer wave IV latency in the CZ-A2 but not the A1-A2 montage. These differences may have arisen from inadequate sampling or sampling bias, since six of eight of the JJ animals came from one litter. The JJ rats were also not age-matched with the other groups; therefore, it is possible that small but significant age-related changes occurring in adult rats gave rise to the differences between the heterozygote and normal controls. Nevertheless, studies showing small neuroanatomic abnormalities in heterozygote Gunn rats[53] make these differences worth pursuing.

Only genotype showed a significant effect on the BAEP; sex and weight did not. Two genotype-by-sex interactions were statistically significant, an unusual finding we cannot explain. The auditory system of the jj Gunn rat, despite abnormal wave amplitudes and latencies, responded normally to rapid rates of stimulation. Unfortunately, the improved yield from high rate of stimulation observed in humans has not yet been verified in rats.

Although we analyzed multiple dependent variables, chance alone would be expected to account for less than one significant comparison at the $p < .005$ significance level, far less than the number of significant two-way ANOVAs observed. The reader is reminded, however, that nonsignificance establishes only that the group differences are not significant for this sample size and variability under these test conditions. Larger samples, smaller variance, or different stimuli might change the significance of specific comparisons, although we believe the overall pattern of results is not likely to change substantially.

To correct for errors of multiple inferences from our data set, we divided the desired overall error rate, α^*, of .05 by the number of inferences (n) to give an individual α criterion for each inference; thus, each individual $\alpha = \alpha^*/n$.[50] So, for example, five inferences at $\alpha = .01$ would give an overall α^* of .05. This procedure, however, assumes independence of the dependent variables, a difficult assumption to make for this data set.

In selecting our α we assumed that each BAEP wave acts as an independent variable, that the A1-A2 and CZ-A2 montages are two independent projections of a three-dimensional event, and that wave latency and amplitude are independent. Thus, for $n = 16$ the individual α value of .003 gives an overall α^* of .05. We realize our assumptions of independence and choice of n are debatable. Therefore, in this initial descriptive study we have chosen to present additional, clearly nonindependent comparisons, including both waves and interwave intervals, amplitudes, and amplitude ratios.

Previous BAEP studies in jj rats have given conflicting results. Normal auditory function[48] and abnormal central auditory function[45] have been found in jj rats. Initially, Levi et al[48] reported normal BAEPs in jj adult rats. However, Uziel et al[45] studied "symptomatic" adult jj rats and found abnormal amplitudes and shape for wave II. Our results are consistent with both central and peripheral auditory dysfunction and thus confirm and extend the findings of Uziel et al.

Because of the small magnitude of most of the abnormalities we report, it is not surprising that previous studies failed to detect them. Earlier studies used smaller numbers of animals and less systematic experimental control of variability. Our data extend the work of Levi et al,[48] who found no abnormalities, and Uziel et al,[45] who found one BAEP abnormality in adult Gunn rats.

In accounting for the small magnitude of the BAEP abnormalities in jaundiced rats, we must consider both the natural selection of less affected animals and the recovery of damaged neurons. Bilirubin encephalopathy is of variable severity in jj rats (as it is in humans), causing death from kernicterus in some animals and very few symptoms in others. The animals more severely affected with bilirubin encephalopathy usually die in the first few weeks of life. It is these animals that have the most severe pathologic changes and are thus likely to have more severe electrophysiologic abnormalities. Obviously, adult studies examine only those less severely affected animals that survive to adulthood. Also by adulthood, functional recovery of previously damaged neurons may occur, as suggested by histologic evidence.[46] Therefore, one reason for the small magnitude of the differences may be the age at which we and others have studied these animals. We have seen more striking BAEP abnormalities in younger, more severely affected jj animals.

Developmental studies are needed to further our understanding of the effect of bilirubin toxicity on the auditory nervous system. Acute, evolving changes, inapparent from adult studies, may occur early in development and shed more light on the pathogenesis of bilirubin toxicity. The changes we observed in the adult jj rat are measured following long-standing hyperbilirubinemia. However, the majority of audiologic damage may occur during the first 2 or 3 weeks of life, when serum bilirubin is at its peak (two to three times higher than adult values) and when the sensory system is immature and perhaps more susceptible to bilirubin toxicity. The normative data collected in this study can be used as a reference for these studies.

The precise definition of the anatomic site of bilirubin-induced injury might lead to the initiation of more effective therapeutic interventions. For example, amplification would be an inappropriate strategy for the hearing-impaired person whose disorder resides in structures central to the cochlea. Similarly, treatment of hyperbilirubinemia may be improved by understanding the temporal course of bilirubin toxicity. Exchange transfusion or phototherapy might be effective therapy initially, but completely ineffective after further damage has occurred. These observations reinforce the need for developmental studies in infant Gunn rats.

In conclusion, the results of this study support both schools of thought regarding the locus of the auditory deficit in bilirubin encephalopathy. There appear to be abnormalities centrally, in the cochlear nuclei and other central auditory brain stem pathways, and peripherally, at or proximal to primary auditory neurons. The peripheral abnormalities may be further resolved by other measures, including cochlear microphonic potentials, single unit recordings from hair cells and auditory nerve fibers, and light and electron microscopy of the cochlea and auditory nerve. Similarly, the central abnormalities could be further resolved by near-field intracranial and longer latency evoked potentials, single-unit recordings in the central auditory nuclei, and histologic and histochemical techniques. Finally, the interaction of bilirubin toxicity with development must be clarified before the pathogenesis of bilirubin toxicity is fully understood.

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IMAGING CASE STUDY OF THE MONTH

COMPUTED TOMOGRAPHY OF INTERNAL JUGULAR VEIN THROMBOSIS

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INTRODUCTION

Venous thrombosis is a process consisting of total coagulation of blood in some predisposed segment of a vein. Ninety percent of cases are encountered in the lower extremities. Venous thrombosis in the head and neck is uncommon unless an obstruction occurs. We present a case of carcinoid tumor in which jugular venous thrombosis was found. Computed tomography provides an accurate diagnosis and prompts immediate therapy.

CASE PRESENTATION

A 73-year-old woman was admitted for right arm swelling and superior vena cava syndrome. Two

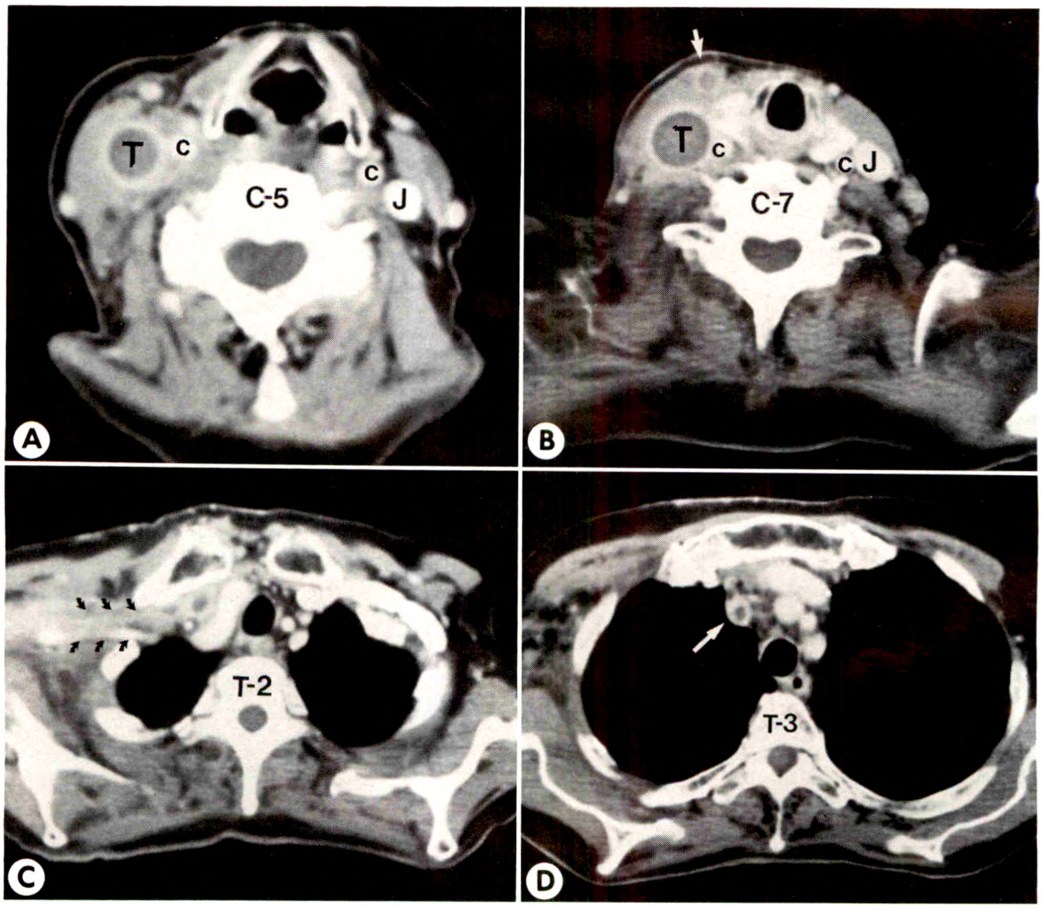

Fig 1. Post-contrast computed tomographic scan of neck. A) At C-5, enlarged right internal jugular vein is seen with enhanced wall and low-density lumen (T) representing thrombosis formation. c — carotid artery, J — internal jugular vein. B) At C-7, small low-density ring lesion is present in superficial cervical vein (white arrow). C) At T-2, dilated thrombosed subclavian vein (arrows) merges with internal jugular vein. D) At T-3, narrowed superior vena cava (white arrow) is seen.

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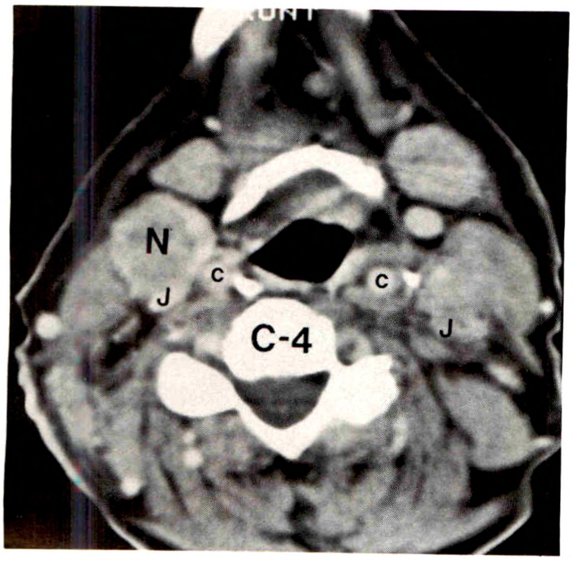

Fig 2. Lymph node enlarged by metastasis. Contrast scan at C-4 shows ring with irregular low-density center (N). Right jugular vein (J) is compressed by node. c — carotid artery.

years prior to admission, she noticed a right lower quadrant pulling sensation and abdominal bloating. This was diagnosed as Crohn's disease and treated with prednisone with improvement of symptoms. About 6 months prior to admission she began having four or five watery stools per day with nocturnal diarrhea and intermittent flushing. Steroid therapy was continued. About 1 month prior to admission she underwent exploratory laparotomy for a small bowel obstruction. Multiple peritoneal seeding with masses involving the ovary and small bowel was found. Biopsy revealed carcinoid tumor. She was referred to a medical oncologist for chemotherapy and was found to have right arm swelling and venous engorgement over the neck and the chest.

A CT scan with contrast showed a narrowing of the superior vena cava at the level of the aortic arch. A well-defined, round, low-density mass with surrounding enhancing rim was seen in the right jugular vein (Fig 1A). Peripheral collateral circulation in the subcutaneous tissue was visible. The thrombus extended from the level of the aortic arch to the angle of the mandible (Fig 1B-D).

The clinical impression was internal jugular vein thrombosis. Anticoagulant treatment with intravenous heparin followed by oral Coumadin began immediately. The patient had rapid relief of swelling within 1 week.

DISCUSSION

Venous thrombosis in the neck is very uncommon. It has been reported in drug abusers or as a complication of central venous catheterization.[1] Another cause is venous compression produced by tumors in the region of the internal jugular vein, such as glomus tumor, neurofibromatosis, neurolemmoma, chordoma, and metastasis. Thrombosis associated with carcinoid tumor has not been reported.

Zerhouni et al[2] described several characteristic CT features of venous thrombosis. These include 1) enlargement of the thrombosed vein, 2) a low-density lumen, and 3) a sharply defined wall. All of these features were seen in our patient, who also showed venous distension in the subcutaneous tissues of the neck and chest wall due to superior vena cava obstruction.

Multiple causes of low-density CT lesions in the neck have been described by Reede et al.[3] The "ring-enhancing" lesion with a low-density center seen in Fig 2 represents a large necrotic lymph node metastasis from a base of tongue squamous cell tumor. The location of this node simulates that of a thrombosed jugular vein; however, close inspection of Fig 2 shows the location of an enhanced, compressed jugular vein. The necrotic lymph node would only be seen on a few CT scans, depending on the size of the node. A thrombosed jugular vein would be seen throughout the length of the vein in the neck. The margin of the ring in a thrombosed vein is very smooth, while the ring of the necrotic lymph node has a very irregular inner border.

Using contrast-enhanced CT scanning, the examiner can easily demonstrate the characteristic features of internal jugular vein thrombosis and clearly define the soft tissue structures of the neck. Computed tomography offers a noninvasive means of evaluating the neck vessels and is safer than angiography. It is probably more accurate than ultrasound or nuclear scans.

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PATHOLOGY CONSULTATION

PARAPHARYNGEAL AND RETROPHARYNGEAL SPACE DISEASES

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The involvement by a disease process of the parapharyngeal and retropharyngeal spaces poses significant diagnostic and therapeutic problems. Neoplasms, the majority histologically benign, comprise the greatest number of parapharyngeal space lesions. Inflammatory lesions and metastases to lymph nodes are the preponderant disorders affecting the retropharyngeal space.

The pharynx is surrounded by several potential spaces, which form a complete ring around it. These spaces lie deep in the superficial or anterior layer of the deep fascia, and they communicate with each other around the muscles and vessels that pass through them.

Neoplasms or other disease processes involving these spaces provide not only diagnostic but also therapeutic challenges. Antibiotics have significantly decreased the formerly serious infections in the spaces, so that neoplastic disorders are the preponderant lesions. Even these are uncommon, with only approximately 0.5% of all head and neck tumors presenting in the parapharyngeal space.

PARAPHARYNGEAL SPACE

The parapharyngeal space proper is roughly in the shape of an inverted pyramid.[1-3] The base of the pyramid is the base of the skull, and its apex is the greater cornu of the hyoid bone. The posterior boundary is the vertebral column and paravertebral muscles. The medial border of this potential space is the superior pharyngeal constrictor muscle and the tonsillar fossa. The lateral boundary is formed, respectively, from anterior to posterior by the internal pterygoid muscle, the inner surface of the ramus of the mandible, the deep lobe of the parotid gland, and the posterior belly of the digastric muscle.

Fascial planes of the parapharyngeal space allow division of the space into compartments. The fascia enveloping the styloid process and its muscles extends anteromedially to merge with the fascia surrounding the tensor veli palatini muscle. This divides the parapharyngeal space into an anterior or prestyloid compartment and a posterior compartment. Fascia from the carotid sheath extends from this plane posterolaterally to the prevertebral fascia and divides the posterior compartment into lateral or retrostyloid and medial or retropharyngeal compartments. These fascial spaces converge just below and lateral to the apex of the fossa of Rosenmüller.

The prestyloid space contains the internal and external pterygoid muscles, the condyle and ramus of the mandible, and the deep lobe of the parotid gland. Also passing through are the internal maxillary artery and the lingual, inferior dental, and auriculotemporal nerves. The lateral nasopharyngeal wall and the fossa of Rosenmüller superiorly, and the tonsillar fossa inferiorly, relate to the medial wall of the prestyloid space. Directly above lies the sphenoid's greater wing with the foramen ovale, foramen spinosum, and access into the temporal fossa. The floor of this compartment is related to the submandibular gland.

Within the retrostyloid space are the internal carotid artery, the internal jugular vein, the four caudal cranial nerves (IX, X, XI, XII), and the cervical sympathetic chain, as well as numerous lymph nodes.

Four hundred seventy-six space-occupying parapharyngeal lesions, according to tissue of origin, are presented in the Table.[1,4-9] Noteworthy are the observations that 1) the majority are histologically benign (70%); 2) 45% of the tumors are of salivary origin (deep lobe of parotid, minor salivary glands, and ectopic salivary tissue in the fascia of the space); and 3) involvement of lymph nodes is usually by a malignant process (metastases or lymphomas).

RETROPHARYNGEAL SPACE

The retropharyngeal space is situated posterior to the pharynx, bounded by the buccopharyngeal fascia in front, the prevertebral fascia behind, and the carotid sheath laterally.[10] The space extends from the base of the skull to the retroesophageal space in the posterior mediastinum. The lower limit is at the level of the sixth thoracic vertebra, where it is

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PARAPHARYNGEAL SPACE LESIONS

Tissue of Origin	*Number of Lesions*		*Percent of Total*
Salivary gland neoplasms		216	45.0
Benign	143		
Malignant	73		
Schwann cell neoplasms		95	20.0
Benign	89		
Malignant	6		
Paraganglionic neoplasms		51	10.7
Benign	50		
Malignant	1		
Non-neuroectodermal soft tissue lesions		37	7.7
Benign	27		
Malignant	10		
Lymphoreticular lesions		41	8.6
Benign	4		
Malignant	37		
Metastases		16	3.4
Branchial cysts		10	2.1
Inflammatory lesions		10	2.1
Total		476	

Data from references 1 and 4 through 9.

closed by fibrous connective tissue about the bifurcation of the trachea.

Lymph nodes and lymphatics are the principal, if not sole, contents of the retropharyngeal space.[10,11] The lymph nodes are aligned in four groups in two longitudinal chains, posterior to the pharyngeal constrictors, on each side of the midline of the pharynx. The lateral groups are constantly present in infants and young children, but in adults, one of the chains is likely to be absent.[11] An inconstant medial group of lymph nodes is found in infants and children. Afferent drainage of the retropharyngeal lymph nodes is primarily from the nasopharynx, paranasal sinuses, soft palate, pharynx, middle ears, and eustachian tubes.[12]

In contrast to the parapharyngeal space, where inflammatory disease and metastases collectively account for only 5.5% of that space's lesions, the preponderant afflictions of the retropharyngeal space are inflammation (abscesses) and metastases to lymph nodes.

Anatomic features facilitate the spread of infection. Only the carotid sheath separates (laterally) the retropharyngeal space from other fascial spaces in the neck. Because of this limited barrier, a retropharyngeal abscess may extend to the parapharyngeal space and vice versa. The continuity with the retroesophageal space allows infection to spread from the neck to the mediastinum. Abscesses, cold or otherwise, also may ensue from inflammatory disease in the prevertebral space.

Retropharyngeal abscesses occur preponderantly in infants and children, and although antibiotics have decreased the incidence, they can give significant morbidity. In children, the source is usually an infection of the sinonasal tract, throat, or middle ear. Secondary involvement also can occur via vascular spread or by a direct inoculation by trauma to the wall of the pharynx. Dental infections, pharyngitis, and trauma are the principal causes in adults.

The actual incidence of metastases to the retropharyngeal lymph nodes is unknown, but is likely quite common, especially from carcinomas of the nasopharynx. Ballantyne[12] indicates an incidence of 44% in 34 patients with cancer of the pharyngeal walls treated by surgery and further relates that the nodes may be a significant route in some other carcinomas of the oropharynx and hypopharynx, particularly the pyriform sinus.

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LETTERS TO THE EDITOR

To the Editor:

In the X-ray Study of the Month (Clary R, Weber AL, Eavey R, Oot RF. Orbital cellulitis with abscess formation caused by sinusitis. *Ann Otol Rhinol Laryngol* 1988;97:211-2), the statement is made: "If there is a progressive decrease in vision along with proptosis (usually indicative of spread or expansion of subperiosteal abscess), surgical intervention is indicated." This statement is correct as it stands, but it may convey the most dangerous impression that one should wait for deterioration in visual acuity before embarking upon surgery. At the Royal Alexandra Hospital for Children, Sydney, Australia, our aim is to provide surgical drainage before deterioration in vision occurs, if at all possible, and thereby try to prevent it. It would seem that the aim of Clary et al is much the same, because in their case, "On the basis of the CT examination [subperiosteal abscess] and the clinical findings, the patient was taken to the operating room . . . ." In particular, they make no mention of the patient's visual acuity in the clinical findings. Apparently the CT examination finding of a medial orbital subperiosteal abscess was sufficient indication for drainage. It should be, without waiting for "progressive decrease in vision," which may be irreversible and which could be a harbinger of death.

Less important, the article contains, in my opinion, two misquotes. First is the statement: "In 60% of patients with acute orbital inflammation, sinusitis has been found to be the cause"; reference is made to an article attributed to Williams-Noble FA (sic). I assume this is the article by Williamson-Noble[1] in which the statement is made on page 51: "Inflammatory disease of the sinuses is the commonest cause of ocular and orbital complications, and Birch-Hirschfeld has estimated that this occurs in two to three per cent. of cases, he also estimated that 60 per cent. of all orbital inflammations were due to paranasal sinusitis. This statement was made in 1909 at a time when the importance of sinus infection in producing orbital disease was not fully recognised and the correct figure is probably 70 per cent., though some observers have put it as low as 39 and others as high as 100 per cent." Clary et al have paraphrased this statement inaccurately.

Secondly, Clary et al make the statement, "Orbital cellulitis can be subdivided into 1) preseptal inflammation, 2) preseptal inflammation with a subperiosteal infiltrate or abscess, 3) orbital cellulitis involving the extraconal and intraconal spaces, and 4) an orbital abscess in the intraconal and extraconal compartments." Reference is made to Chandler et al.[2] I have reread this article by Chandler et al and confirmed my memory that those authors proposed a method of classification of the local orbital complications of sinusitis into group I, inflammatory edema; group II, orbital cellulitis; group III, subperiosteal abscess; group IV, orbital abscess; and group V, cavernous sinus thrombosis; they made no mention of the extraconal and intraconal spaces. While the division of orbital inflammations, which Clary et al have presented is not unreasonable, it is not what Chandler et al proposed and it may confuse thought on the topic. If Clary et al are quoting another classification they should refer to it, not inaccurately to Chandler et al; if they are proposing a new one they should say so.

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To the Editor:

Our thanks are extended to Dr Harrison for reading our article with scrutiny. Of course, we agree that loss of visual acuity (which, by the way, could not be tested satisfactorily in this particular patient) is a late indication. It is, however, an absolute indication, and was included only for completeness in this article that emphasizes radiologic findings. We agree that CT scan findings can be and were in this instance the primary influence for surgical decision-making.

Regarding Dr Harrison's other comments, the quote "60%" probably does not paraphrase the original statement adequately, but is consistent with other references (Chandler's) to this paper. Also, the reference to Chandler's paper was in regard to the pathogenesis of orbital inflammation and should have been placed after the sentence preceding the one to which it was attached. The orbital cellulitis classification found in this paper was derived from the experiences of Dr Weber (a coauthor of our article). We appreciate Dr Harrison's careful detection of these discrepancies.

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BOOK REVIEW

Rehabilitative Audiology: Children and Adults

Jerome G. Alpiner and Patricia A. McCarthy, editors. Hard cover, illustrated, indexed, 533 pages, 1987. Williams & Wilkins, Baltimore, $36.50.

This book provides an up-to-date discussion of a variety of aspects of auditory rehabilitation. Section 1, on identification and evaluation, contains chapters on an overview of rehabilitative audiology, identification of hearing loss in infancy and early childhood, evaluation of adult communication function, and amplification systems for the hearing impaired. Section 2, on the rehabilitation of the hearing-impaired child, contains chapters on speech-language assessment and intervention with preschool hearing-impaired children, speech-language assessment and intervention with school-age hearing-impaired children, education management of the hearing-impaired child, and psychosocial aspects of hearing loss in children. Section 3, on the rehabilitation of hearing-impaired adults, contains chapters on adult remediation process, psychosocial and counseling aspects of the adult remediation process, rehabilitation of the hearing-impaired geriatric client, and the role of ancillary personnel in a multidisciplinary approach. Section 4 discusses the recent developments in rehabilitative audiology.

All the chapters are generally of high quality, up-to-date, and comprehensive. The book is a reference for students in otolaryngology. The appendices could be very helpful. They contain hearing handicap questionnaires, teacher questionnaires, speech-perception tests, lists of organizations having information about managing hearing loss, and information about assistive listening devices.

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INSTRUCTIONS TO AUTHORS

Send manuscripts to Editor Brian F. McCabe, MD, Department of Otolaryngology-Head and Neck Surgery, University of Iowa Hospitals, Iowa City, IA 52242. Original manuscripts dealing with clinical and scientific aspects of otolaryngology, bronchoesophagology, head and neck, maxillofacial and plastic surgery, audiology, speech pathology, and related specialties will be considered for publication.

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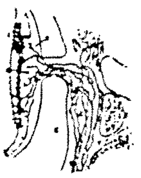

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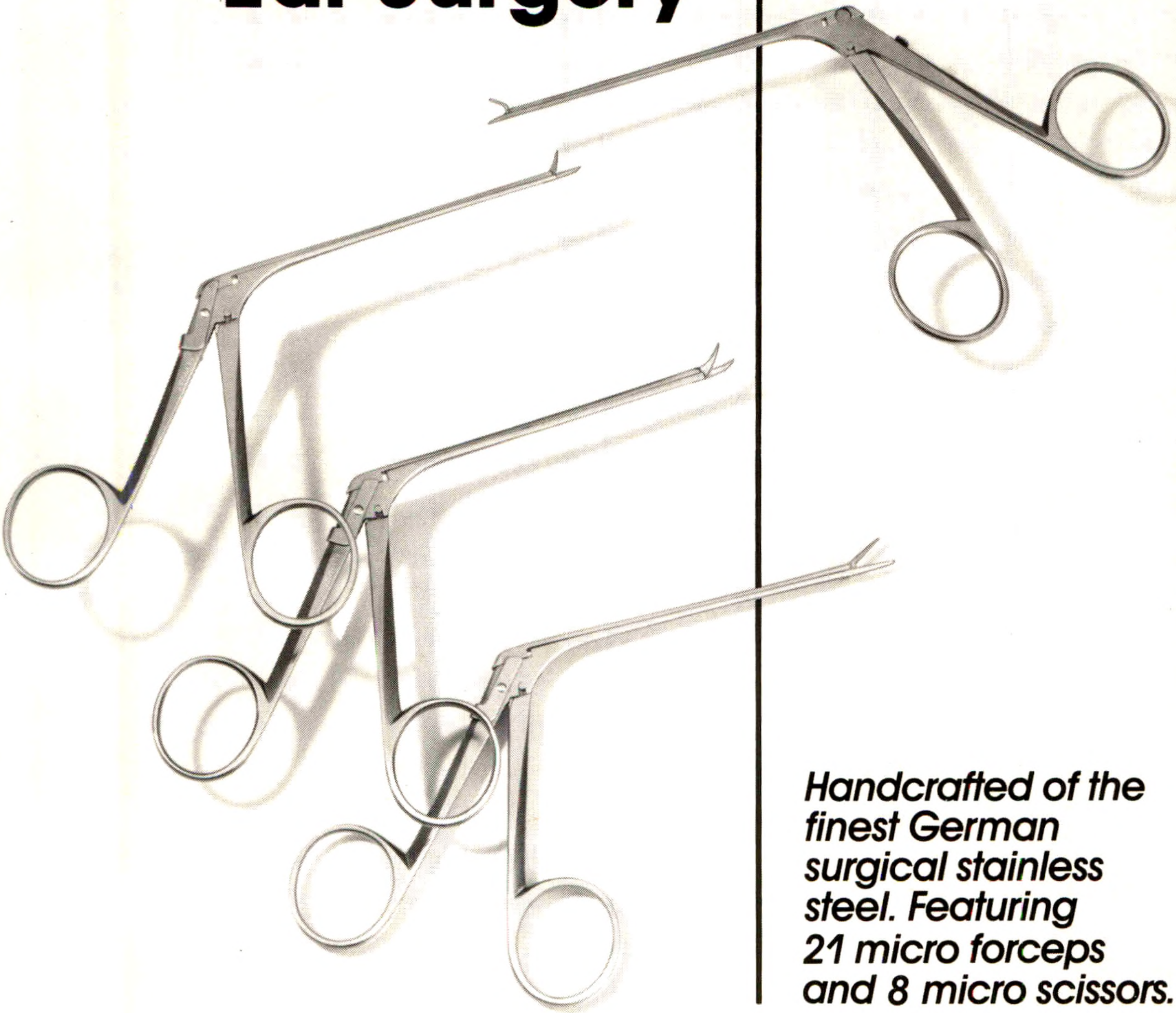

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